THE REGULATION AND METABOLISM OF GLUCOSE IN THE GREEN ANOLE (*ANOLIS CAROLINENSIS*)

By

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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The ways in which glucose is regulated and metabolized in the green anole, *Anolis carolinensis*, were examined and compared with mammalian systems. Parameters examined were seasonal variation in stored glucose, regulatory enzyme activities associated with glucose metabolism and the effects of exercise, fasting, insulin, glucagon and carbohydrate loading on glucose.

Plasma glucose and tissue glycogen show seasonal variation. Plasma glucose levels are highest in the spring and lowest in late summer and early fall. Muscle and liver glycogen levels peak during winter and are lowest during the summer. The low levels of tissue glycogen and plasma glucose during the summer coincide with the reproductive season of the green anole.

The decrease in glucose during the breeding season may be due to the influence of reproductive hormones on the behavior of the lizards, and the increased catabolism of glucose.

Anaerobic enzyme activities with the exception of hexokinase are higher in *Anolis carolinensis* than in mammals. Mitochondrial enzyme activities in *Anolis* are lower than those in mammalian mitochondrial enzymes. The lower mitochondrial activities in the lizard are probably due to lower mitochondrial volume densities and mitochondrial membrane surface areas. These factors are correlated with differences in the metabolic rates of the two groups and the reliance of *Anolis* on anaerobiosis to support burst activity.

The most probable fate of lactate produced during exercise in *Anolis* is gluconeogenesis. Recovery from lactate acidosis occurs at a faster rate than tissue glycogen repletion, and excess glucose is removed preferentially by the liver after exercise. Fasting depresses tissue glycogen levels, but does not affect the manner in which glucose is used.

Carbohydrate loading in resting animals produced long lasting hyperglycemia. Relatively high insulin concentrations are required to elicit hypoglycemia, which is of short duration and is followed by a compensatory hyperglycemia. Hyperglycemia is probably due to glucagon secretion.

CHAPTER 1
BACKGROUND AND PERSPECTIVE

The Problem

Vertebrate ectothermic poikilotherms rely principally on anerobic metabolism to meet the energy demands of intense activity. Reliance on anaerobic pathways reduce the ability to maintain long episodes of intense activity because of the production of large amounts of lactate (Bennett 1978, Bennett et al. 1981, Gleeson 1980a, 1980b, 1982, Gaesser and Brooks 1984, Gratz and Hutchison 1977, Hutchison et al. 1977, Hutchison and Turney 1975, Loumbourdis and Hailey 1985, Putnam 1979a, 1979b, Warnock et al. 1965). The increase in blood and tissue lactate concentrations may be as high as 10 times resting levels after prolonged activity and may cause major pertubations in blood and muscle pH (Bennett 1973, Bennett and Licht 1972, 1973). A decrease in the oxygen transporting ability of blood as well as rates of enzyme catalysis may occur as a result of lactate production.

Metabolic products other than lactate formed during anaerobiosis presumably are not important in the synthesis of high energy phosphate compounds in vertebrate ectotherms. Removal of excess blood lactate produced during activity may take several hours (Gleeson 1980a, 1980b).

Repayment of oxygen debt is usually much shorter than the time for lactate reduction (Brooks et al. 1973, Gaesser and Brooks 1984, Gleeson 1980, Gratz and Hutchison 1977). In mammals, lactate is rapidly oxidized to carbon dioxide and water (Brooks et al. 1973, Drury and Wick 1956). The fate of anaerobically produced lactate is not known in reptiles. Although muscle and liver glycogen levels are reestablished, the location of lactate catabolism is not known. The skeletal muscle of amphibians have shown some ability to oxidize lactate (Bendall and Taylor 1970, Gourley and Suh 1967). One potential site of lactate catabolism is the liver. Relatively high specific activities of lactate dehydrogenase have been found in this tissue in reptiles (Baldwin and Seymour 1977).

Due to reliance on glycolysis, the regulation of glucose levels must be of physiological importance in ectotherms. Most studies concerning anaerobiosis in terrestrial ectothermic vertebrates focus on either lactate production or muscle glycogen utilization (Bennett 1978, Bennett and Gorman 1981, Bennett and Ruben 1975, Callard et al. 1975, Coulson and Hernandez 1953, Gleeson 1980a, 1980b, Loumbourdis and Hailey 1985, Pough and Andrews 1985, Putnam 1979a, 1979b). Few studies examine both lactate production and glucose use simultaneously (Gleeson 1982, 1986).

The role of liver glycogen during and after activity is not known. Liver glycogen stores of some ectothermic vertebrates may increase or decrease during bouts of exercise (Dean and Goodnight 1964, Gleeson 1982, Gratz and Hutchison 1977). According to one study liver glycogen is not an important source of energy during and after activity (Gratz and Hutchison 1977).

In mammals regulation of blood glucose levels is important to maintain brain function. Approximately one-half of all the glucose formed by gluconeogenesis during interfeeding periods is used for brain metabolism (Guyton 1971). Most reptiles and birds possess blood glucose levels significantly higher than mammals; however, among the reptiles there is considerable variation in levels of blood glucose (Epple and Lewis 1973, Dessauer 1970, Penhos and Ramey 1973, Umminger 1977). Lizards appear to possess the highest levels, with concentrations over 200 mg % being measured (Callard and Chan 1972, Callard et al. 1975, Haggag et al. 1966, Umminger 1977, Zain-ul-abedin and Qazi 1965). Lower levels are found in other reptilian groups (Algauhari 1967, Coulson and Hernandez 1953, Dessauer 1970, Epple and Lewis 1973, Gratz and Hutchison 1977, Houssay and Penhos 1960, Hutton 1958, Penhos and Ramey 1973, Skoczylas and Sidorkiewicz 1974, Umminger 1977, Zain-ul-abedin and Qazi 1965). During exercise blood glucose may increase or decrease (Bennett 1978). In one case where glucose increased

during and after exercise, it remained high for several hours during recovery while liver levels remained unchanged (Gratz and Hutchison 1977). The source of this increase in blood glucose is not known. One possibility is gluconeogenesis from lactate via the Cori cycle.

Several factors may contribute to the maintenance of high blood glucose in reptiles. First, reptiles, as with mammals, may maintain high levels of glucose for brain function. Second, reptiles may maintain relatively high levels of blood glucose because of their heavy reliance on anaerobiosis. Free glucose in the blood can serve as additional storage. In this form glucose is available for sudden bursts of activity but is not critical for resting metabolism. Third, high plasma glucose may be due to an inherently low insulin concentration in the blood or low production by the pancreas. The latter has been shown in birds (Hazelwood 1973).

The most obvious effect of insulin given to a mammal is a rapid decrease in the blood glucose level. This decrease is a consequence of the enhanced transport of glucose across cellular membranes. In addition to causing a drop in blood glucose levels, insulin has several secondary effects including the indirect activation of glycogen synthetase. In terrestrial ectothermic vertebrates the effect of insulin is not as clear. In the snake Psammophis sibilans, insulin has no effect except when injected with very high dosages (Algauhari 1966).

Even with these high dosages a lowering of blood sugar is only seen 10 hours after the injection. In Rana pipiens, insulin stimulates the uptake of glucose into cells and the synthesis of glycogen (Gourley and Suh 1967, 1969). It also accelerates glycolysis through an enhancement of the activity of phosphofructokinase (Ozand and Narahara 1964). Rana catesbeiana, however, has a very slow response to exogenous insulin (Wright 1959).

High levels of glycolytic enzymes and regulation of those that are rate limiting are adaptations allowing for high rates of glycolytic pathways in vertebrates (Baldwin and Seymour 1977). Hexokinase and phosphofructokinase catalyze irreversible steps in glycolysis, and phosphofructokinase is the rate limiting enzyme. Phosphorylase is important in the sequence through which glycogen enters glycolysis. Because glycogen is the main substrate for anaerobically active muscle tissue, this enzyme plays a vital role in the production of adenosine triphosphate (ATP). The activities of these enzymes provide a measure of the maximum rate of glucose breakdown anaerobically (Baldwin et al. 1977, Bennett 1974, Crabtree and Newsholme 1972, Ozand and Narahara 1964). Low levels of hexokinase have been measured in those reptiles examined, which may indicate they are unable to utilize glucose very rapidly as an energy source (Baldwin and Seymour 1977).

The nutritional state of an animal should, in part, be responsible for the level of energy reserves found in the body. Many ectotherms become inactive during part of their annual cycle. The animal must rely on its energy reserves during this temporal inactivity. Certain ectotherms show resting body glycogen levels that vary 100% or more seasonally (Gleeson 1982, Moore 1967, Patterson 1978, Smith 1954). In other reptiles little seasonal variation occurs (Skoczylas and Siedorkiewicz 1974). In some reptiles fasting has no effect on blood glucose levels for up to 20 weeks (Algauhari 1967). Carbohydrate digestion leads to increased glycogenesis in some lizards (Gleeson 1982). Glucose present after exercise is polymerized mainly in the liver where the amount of glycogen stored exceeds the pre-exercise levels (overcompensation). Overcompensation occurs in muscle only if a second glucose feeding is given during recovery (Gleeson 1982), which is similar to the response seen in mammals (Bergstrom et al. 1967, Gaesser and Brooks 1980, Pernow and Saltin 1971).

Because anaerobiosis has such an important role in the metabolism of activity in ectothermic vertebrate the following questions arise. What is the specific role of glucose in anaerobiosis, and how is it utilized in these organisms? Is blood glucose and/or tissue glycogen regulated as in higher vertebrates? If so what is the

regulating mechanism(s)? Is body glucose a limiting factor in the metabolism of activity in reptiles? If so, how?

Approach

This study examines ways in which glucose may be regulated, metabolized and stored in a terrestrial vertebrate ectotherm, the green anole, *Anolis carolinensis*. This species will serve as a model system for glucose utilization in a reptile and may then be used for comparison with other vertebrates, ectothermic and endothermic.

The study animal is a representative of one of the largest lizard families in the world. It is typical in many regards to other members of its family and species found in other families. The effects of activity are a major portion of this study. A lizard species was therefore chosen as the study animal because of all reptiles, lacertilians tend to lead more active lifestyles. If glucose is a limiting factor in the metabolism of activity, a lizard species is a prime candidate for this study.

This study is organized into six chapters. Chapter II deals with seasonal variation in blood glucose, skeletal muscle and liver glycogen levels. Chapter III presents the activities of various anaerobic and aerobic

regulatory enzymes. These activities are compared to those measured in mammals. Chapter IV examines the effect of exercise on tissue glycogen and blood glucose levels. Chapter IV also details the effect of carbohydrate loading and the relationship between it and glycogen The production and removal of lactate is also examined in Chapter IV. Chapter V looks at the effect of insulin and glucagon on blood glucose and tissue glycogen. Chapter VI is a summary of all findings and presents a model system of glucose regulation and utilization based on all the interrelationships presented in the previous chapters.

CHAPTER II
SEASONAL VARIATION IN BLOOD GLUCOSE AND GLYCOGEN STORES

Introduction

An analysis of any physiological function that is affected by a number of exogenous factors requires information on the seasonal variability inherent in that process. Data on seasonal variation in body glucose levels in reptiles is sparse (Dessauer 1953, Moore 1967). Here I characterize seasonal variability in plasma glucose and/or tissue glycogen. These data shall form the basis for all further experimentation in the later chapters.

Methods

Green anoles of both sexes were collected monthly (n = 7), around Gainesville, Florida, from December 1984 to April 1987. They were returned to the laboratory after capture. The lizards were kept in a constant temperature cabinet set at the ambient temperature at which the animals were captured for 24 hours. After the 24-hour acclimation period, blood and tissue samples were taken and analyzed. All sampling was done between 10:00 a.m.

and 12:00 p.m. E.S.T., which corresponds to the times of capture on the previous day. Others were obtained by purchased from an animal dealer in Louisiana when the numbers of lizards captured locally were small. Blood samples were collected in heparinized capillary tubes after decapitation. The samples were then centrifuged on a micropipette centrifuge for 3 minutes. Plasma glucose concentration was measured colorimetrically using Glucostat test kits (Worthington Biochemical Corporation) and a model 6/20 Coleman Junior 2 spectrophotometer set at 500 nm. Plasma glucose concentrations are expressed as mg/dl or mg%. Different animals from those used for plasma glucose were used for liver and muscle glycogen analyses to avoid any problem associated with struggling. To quickly immobilize the animals, they were plunged into liquid nitrogen and stored at -5°C until assayed.

To assay for tissue glycogen, the liver was dissected free along with the musculature of the fore and hind limbs excluding the manus and the pes. The skeletal muscle samples from each animal were combined (fore and hind limb) and analyzed as a unit. All tissues were ground using a chilled mortar and pestle. The tissues were weighed to the nearest 0.001 gram. Glycogen was extracted in hot KOH and hydrolyzed to glucose by the Good et al. method (1933). The glucose

was then measured using the phenol-sulfuric acid method (Montgomery 1957). The tissue glycogen levels are expressed as milligrams glycogen per gram tissue (mg/g). All means are expressed with their standard errors.

Results

Data on seasonal variation in plasma glucose and tissue glycogen are summarized in Figures II-1 and II-2. Plasma glucose levels are highest during the months of April (223 ± 6.9 mg/dl) and May (230 ± 7.5 mg/dl). They are lowest during August (125 ± 3.1 mg/dl). Muscle glycogen concentrations peak during November (5.3 ± 0.06 mg/g) and are lowest during July (3.9 ± 0.03 mg/g). Liver glycogen concentrations are highest during November (38.6 ± 1.06 mg/g) and lowest during August (4.9 ± 0.53 mg/g). In all three cases the differences between the highest and lowest concentrations are significant at the 0.05 level (small sample two tailed T-test).

There are no significant correlations between plasma glucose levels and muscle glycogen levels, or plasma glucose levels and liver glycogen levels (Table II-1). A significant positive correlation occurs between muscle and liver glycogen concentrations (Table II-1).

Figure II-1. Seasonal variation in plasma glucose. Abcissa is labeled with the first letter of each month. December 1985 was omitted for lack of data. The vertical bars are one standard error.

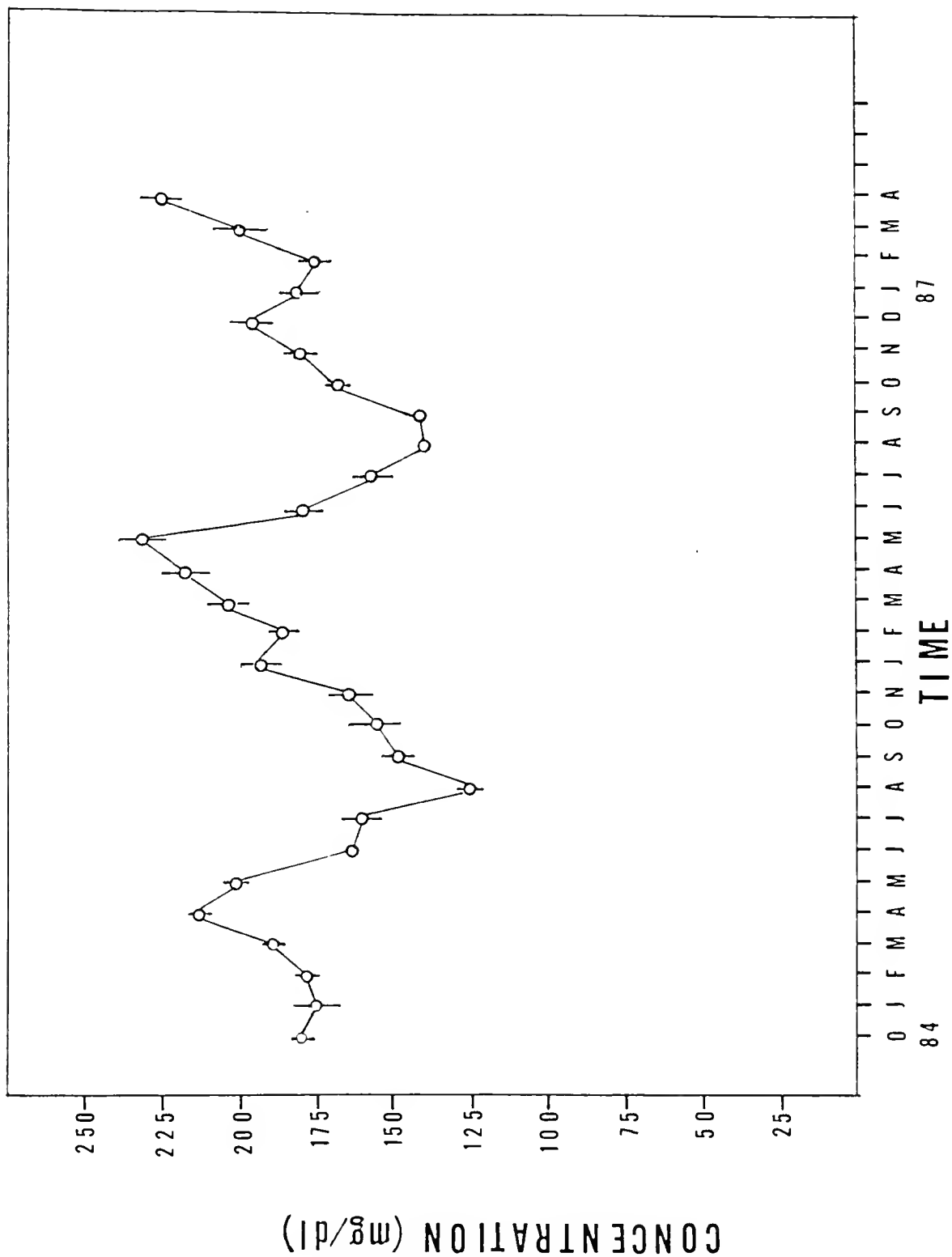

CONCENTRATION (mg/dl)
250
225
200
175
150
125
100
75
50
25
O J F M A M J J A S O N D J F M A M J J A S O N D J F M A
84
87
TIME

Figure II-2. Seasonal variation in liver and limb musculature glycogen levels. Squares = muscle glycogen, circles = liver glycogen. The vertical bars are one standard error.

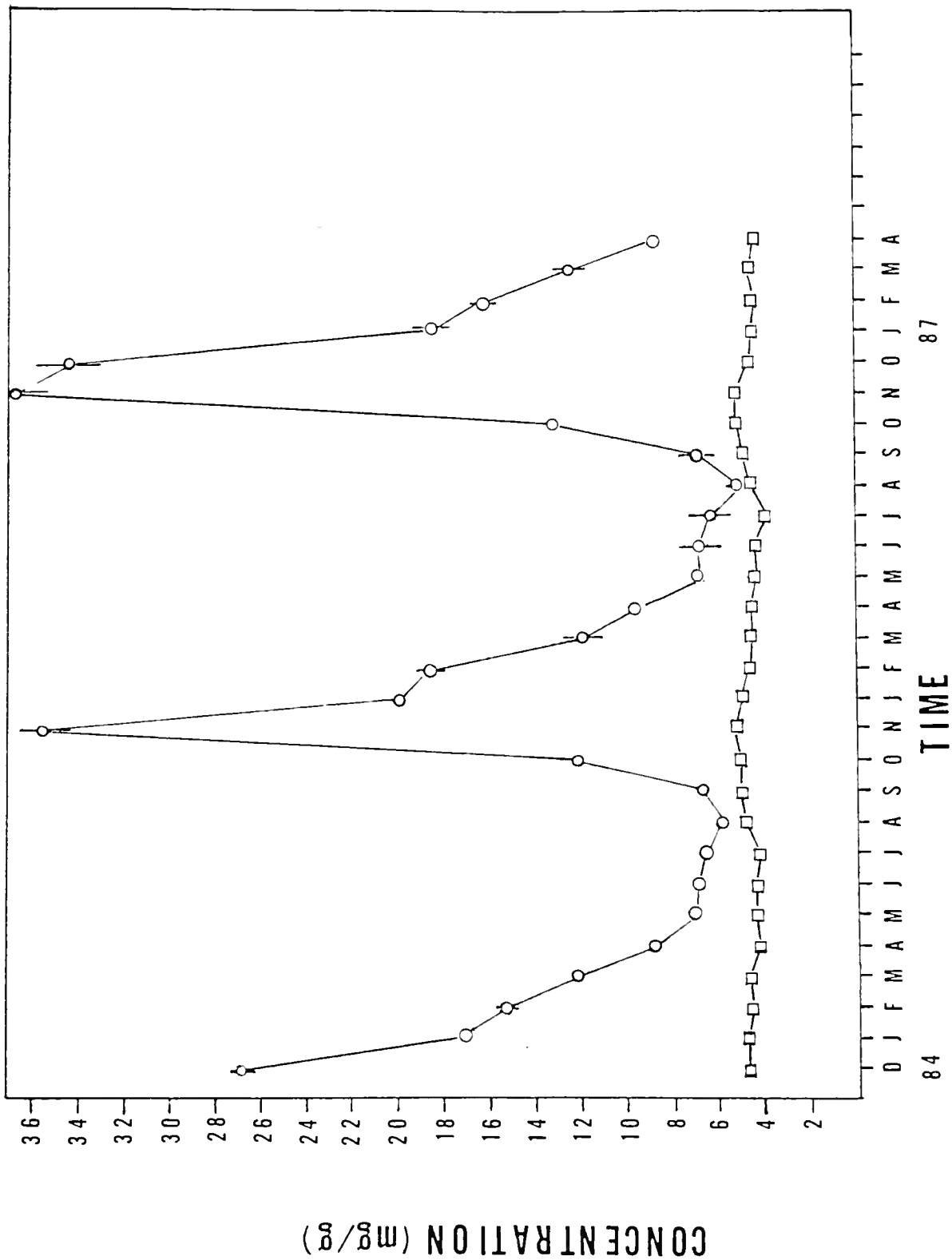

CONCENTRATION (mg/g)
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
D J F M A M J J A S O N J F M A M J J A S O N O J F M A
84
87
TIME

Table II-1. Statistical correlations of seasonal data. Accepted level of confidence equals 0.05.

Correlations	r	P value
plasma glucose vs muscle glycogen	-0.28	> 0.05
plasma glucose vs liver glycogen	0.049	0.40
liver glycogen vs muscle glycogen	0.567	0.001

Discussion

These data show that season affects both plasma glucose and tissue glycogen concentrations. The most marked effect is seen in the liver glycogen levels with an almost 8-fold difference existing between the highest and lowest levels. Plasma glucose and muscle glycogen levels also follow a seasonal trend, although with less variation than liver glycogen.

Liver and muscle glycogen levels appear somewhat in synchrony (Figure II-2). These seasonal data follow the same pattern as seen in other lizards including *Anolis carolinensis* (Dessauer 1953, Gleeson 1982, Patterson et al. 1978). During late fall both muscle and liver glycogen levels begin to increase. This is probably in preparation for overwintering similar to that seen in temperate zone mammals. However, the genus *Anolis* is mainly tropical in its distribution. The species *carolinensis* has the most northern range of the genus, extending up to North Carolina, Tennessee, Arkansas and southeast Oklahoma (Conant 1975). These animals are known to emerge on warm days during the winter over part of their range. Therefore, anoles probably are not true hibernators as are other sympatric lizard species, which may reflect tropical affinities.

Low environmental temperatures may cause a reduced rate of plasma glucose utilization due to the concomitant reduction of metabolic activity (Coulson and Hernandez 1953, Dimaggio and Dessauer 1963). Although plasma glucose levels increase during late autumn through winter, liver glycogen levels start to decline during winter. This suggests that hepatic glycogenolysis is occurring and the free glucose is entering the blood stream. Rapatz and Musacchia (1957) found slight changes in blood glucose but extensive hepatic glycogenolysis in turtles held at low ambient temperatures for 2 months. Blood glucose levels have been reported to increase in amphibians and lizards maintained at low temperatures (Miller 1961, Moore 1967). Increases in blood glucose during winter may be due to a release of glucose by the liver and the body not catabolizing it due to a decreased metabolic rate. Peak levels of hepatic glycogen are almost 8 times higher than the lowest values. Even though a large increase in carbohydrate storage occurs during the late fall, this represents only a small fraction of the total reserve energy. Using standard caloric values, Dessauer (1953) calculated that the stored energy contributed by carbohydrate reserves amounts to only about 3% of the total storage. Similar results were reported for Lacerta vivipara (Patterson et al. 1978). In these two species

the amount of stored glucose is too small to be a significant source of energy during cold torpor; however, tissue glycogen may act as an important source of glucosyl residues (Patterson et al. 1978). If so, tissue glycogen would tend to reduce the catabolism of amino acids which are the last resort to supply glucosyl residues.

Blood glucose continues to rise in the spring, which may be due to carbohydrate intake through feeding. In amphibians no correlation exists between feeding and blood glucose concentration (Wright 1959). However, in Eumeces obsoletus blood glucose concentrations increase with an increase in feeding (Miller and Wurster 1958). In Anolis during the summer, when temperatures are highest and presumably the animals are operating at their eccritic temperatures, blood glucose levels are declining. One would expect that feeding during this time would be as high or higher as during the spring, which may be the case with female lizards, but not in males.

Anolis is typical of other small iguanid species in its reproductive behavior. Males are territorial and actively defend territories against other males (Greenberg and Nobel 1944). Active defense in many cases may preclude other activities, such as feeding, and can be metabolically taxing (Pough and Andrews 1985). From late winter through early autumn liver glycogen reserves are

low (Figure II-2). This period overlaps the reproductive season of Anolis carolinensis (Hamlett 1952). The increase in male-male aggression and territoriality during the breeding season probably reflect the increase in testoterone secretion by the gonads (Crews 1979). Androgen injections are known to produce typical sexual behavior in castrated male anoles (Adkins and Schlesinger 1979, Mason and Adkins 1976).

Females are not territorial and any increase in activity during the reproductive season may simply reflect the higher ambient temperatures during this time of the year. Because no differences were found between males and females in their blood glucose and tissue glycogen levels, something obviously reduces female liver glycogen and blood glucose during the reproductive season. The answer may also be attributable to hormones. Anolis carolinensis has a very peculiar form of ovulation, termed monoallochronic ovulation (Smith et al. 1973). Females ovulate a single egg alternately from each ovary approximately every 14 days during the breeding season (Hamlett 1952, Jones et al. 1983, Nobel and Greenberg 1941). Each egg is oviposited 18 or 19 days after ovulation (Hamlett 1952). Therefore a period of 4 or 5 days exists when one oviduct contains a mature shelled egg while the other oviduct contains a recently

ovulated unshelled egg. During the reproductive season plasma concentration of estradiol-17 beta (E-2) and progesterone are high (Jones et al. 1983). Marschall and Gist (1973) reported reductions in both plasma glucose and liver glycogen levels following estradiol and follicle stimulating hormone (FSH) administration. They suggested that mobilization of hepatic glycogen and blood glucose utilization following hormonal treatments is due to the metabolism of carbohydrates contributing part of the energy required for the synthesis of yolk proteins.

Reducing blood glucose may also have secondary effects on other forms of stored energy, such as lipids. In mammals lipid degradation can result from hormone sensitive lipases or may depend on the availability of glucose. Glucose is required for the reesterification of free fatty acids through the generation of glycerol-3-phosphate. Therefore, if glucose availability to the adipose cells is reduced, it results in the release of free fatty acids (Marschall and Gist 1973). Estrogen treatment results in fatty acid release in Uta stansburiana (Hahn and Tinkle 1965). A mechanism for triacylglycerol degradation involving plasma glucose reduction in A. carolinensis may occur. Such a mechanism was suggested by Marschall and Gist (1973) because even when presented with a glucose load, the incorporation of labeled glucose carbon into fat bodies was reduced during estrogen treatment.

CHAPTER III
ACTIVITIES OF REGULATORY METABOLIC ENZYMES

Introduction

Carbohydrates usually make up a large proportion of the diet of most animals. Some of the carbohydrate intake may be converted to, and metabolized as, fat. However the major function of carbohydrates is a source of energy through cellular oxidation. The major form of carbohydrate for oxidation is the hexose glucose. Other principal monosaccharides which may be utilized include fructose, the major form found in fruits, and galactose, which is found in dairy products. Fructose is only important if the diet includes large amounts of sucrose, a disaccharide composed of glucose and fructose. Both fructose and galactose can be converted into glucose through hepatic mechanisms.

Carbohydrate metabolism can occur through several pathways (Figure III-1). The major pathway during activity in reptiles is the oxidation of glucose or glycogen to lactate by the Embden-Meyerhof pathway (Figure III-2). High levels of glycolytic enzymes and regulation of those that are rate limiting are adaptations

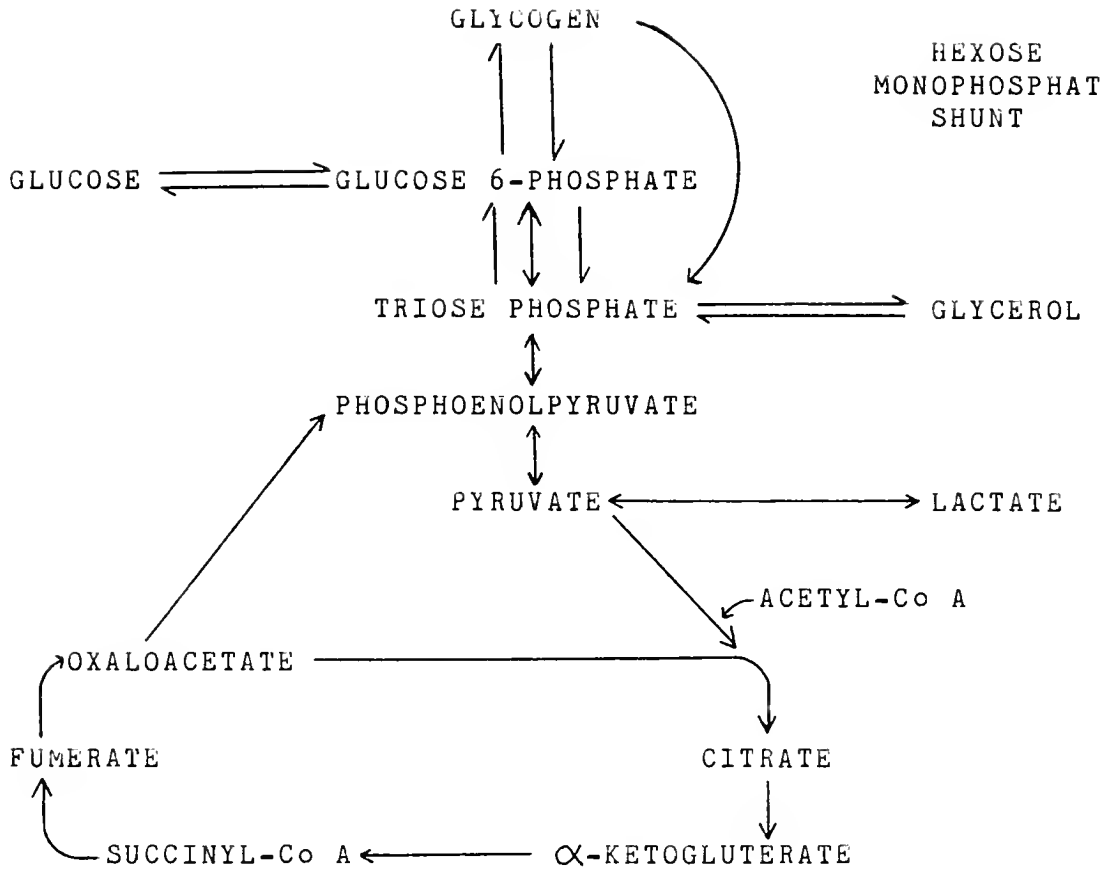


Figure III-1. Schematic of pathways of glucose and glycogen catabolism. Double headed arrows indicate reversible reactions utilizing the same enzyme. Multiple arrows indicate multiple pathways.

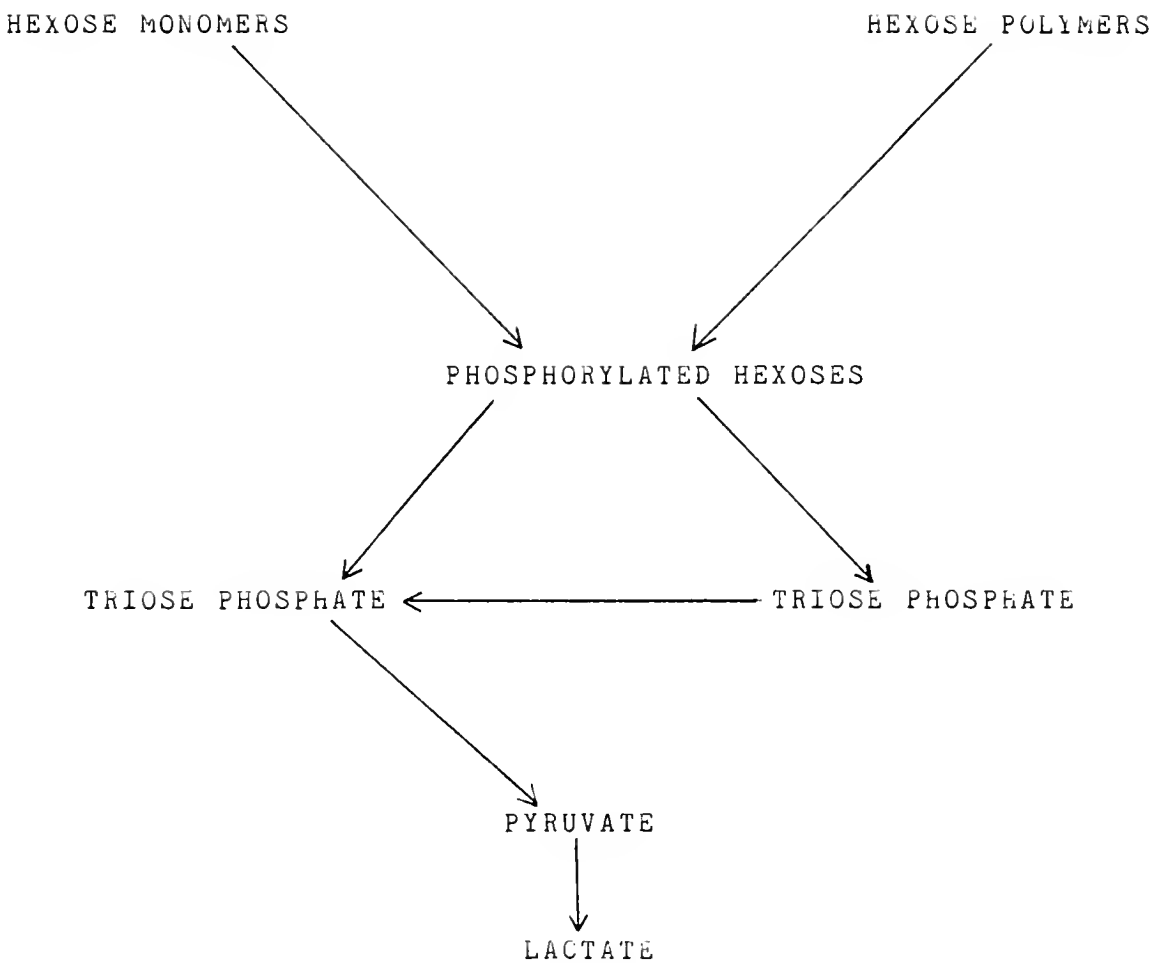


Figure III-2. Simplified schematic of the Embden-Meyerhof pathway.

allowing for high rates of substrate passage through glycolytic pathways. If anaerobiosis represents the major pathway for energy production during activity, one would expect to find high activities of anaerobic enzymes.

Enzymes examined were hexokinase, phosphorylase, lactate dehydrogenase and phosphofructokinase, key regulatory catalysts in glycolysis. These four enzymes are used to estimate the maximum rates of glycolysis in muscle (Crabtree and Newsholme 1972). Phosphorylase is not an enzyme of the Embden-Meyerhof pathway proper (Figure III-3). Phosphorylase is involved in the degradation of glycogen through the mobilization of glucose-1-phosphate. Hexokinase controls the entry of free glucose into the glycolytic pathway through the phosphorylation of glucose. Under physiological conditions this reaction can be considered to be irreversible because of the considerable loss of free energy as heat. Although lactate dehydrogenase is not a rate-limiting enzyme, high levels are usually associated with high anaerobic scopes. These four enzymes are all found in the soluble fraction (cytosol) of the cell.

The mitochondrial enzymes, nicotinamide adenine dinucleotide (NAD)-linked isocitrate dehydrogenase and succinic dehydrogenase are both involved in aerobic metabolism and the generation of electron transporting

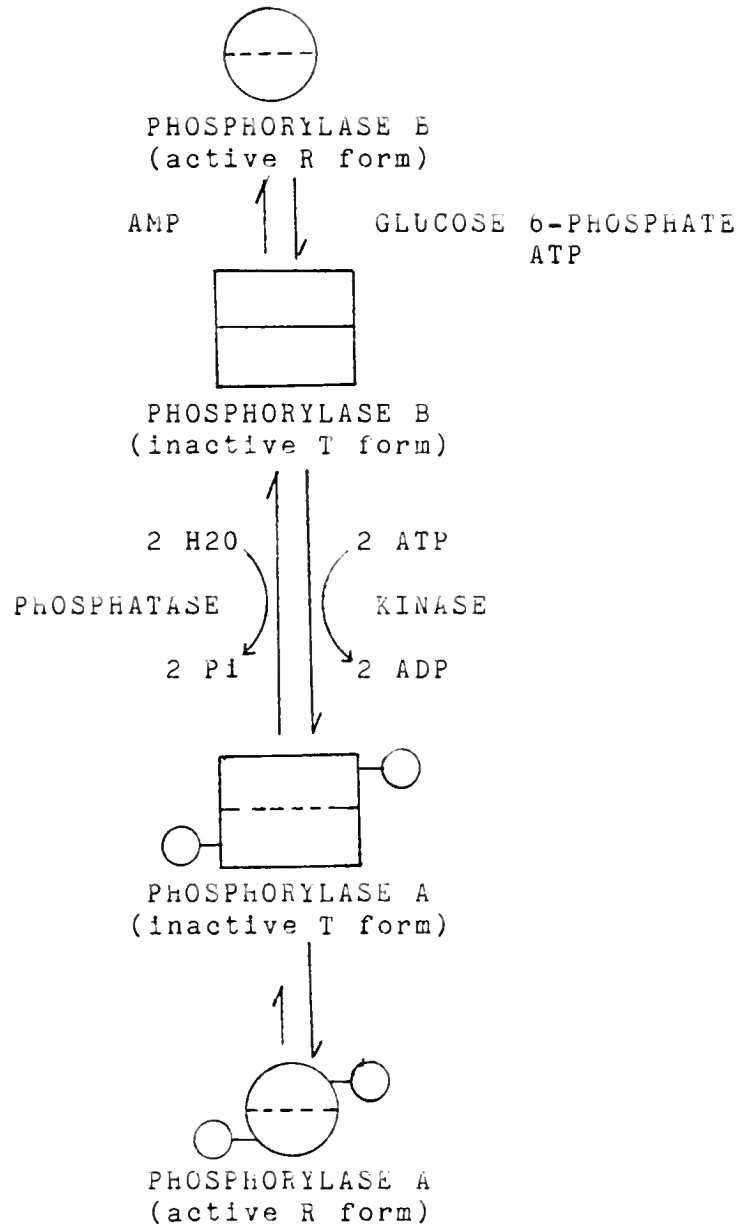


Figure III-3. Control of the activity of phosphorylase in hepatic and skeletal muscle tissue. The enzyme can change from an inactive T (tense) form, to active R (relaxed) form. The proportion of the active conformation is determined mainly by the rates of phosphorylation and dephosphorylation. Adapted from Stryer 1981.

molecules. Thus, both are linked to the production of ATP via the Kreb's cycle and they are also key regulatory steps in the Kreb's cycle.

Methods

Green anoles were obtained from a dealer in Louisiana and held in captivity for 7 days in a thermal gradient (22 - 50 ^{o}C) on a 12:12 light and dark cycle. The lizards were fed crickets and mealworms daily. Five animals were used to measure each enzyme activity. Animals were fasted for 48 hours prior to being assayed. Lizards were killed by freezing in liquid nitrogen. After freezing the lizards were assayed immediately or stored frozen until assayed at a later date. To assay, the entire liver was removed along with the musculature of both fore and hind limbs. All the dissected tissue was weighed and placed in an ice bath. All preparations were performed in an ice bath.

Muscle and liver samples were homogenized separately in ice cold buffered Ringer's solution (1 gram tissue in 4 ml solution, Guillette 1982) with a mortar and pestle. Triton X-100 was added to give a final concentration of 1%. Triton X-100 increases the soluble hexokinase activity (Burleigh and Schimke 1969). After the Triton X-100 addition, the homogenate was incubated on ice for 30 minutes followed by centrifugation at 17000 rpms at 4 ^{o}C.

Enzyme activities were measured according to the methods outlined for each enzyme. Concentrations are expressed in the total volume of the final reaction mixture. All measurements were made with a model 6/20 Coleman Junior 2 spectrophotometer. Samples were kept at 33 ^{o} C (eccritic temperature) in a water bath and the optical density measured every 60 seconds. Mean specific activities are expressed in n-moles of product formed per minute per milligram protein nitrogen. Protein was estimated by the biuret method according to Gornall et al. (1949). Purified bovine serum aloumin solutions were used as standards (Sigma Chemical Company). All means are given with their standard errors.

Hexokinase (E.C.2.7.1.1)

The reaction mixture contained 0.75 mM NADP, 3 mM ATP, 7.5 mM magnesium chloride, 0.2 mM glucose, 1.5mM potassium chloride, 2 IU glucose-6-phosphate dehydrogenase, 5 mM mercaptoethanol, and 50 mM sodium phosphate buffer pH 7.4 (Baldwin and Seymour 1977). The reaction was started with the addition of homogenate. Enzyme activity was measured by following the reduction of NADP at 340 nm.

Lactate Dehydrogenase (E.C.1.1.1.27)

The reaction mixture contained 33 mM phosphate buffer pH 7.4, 0.067 mM NADH, 0.33 mM sodium pyruvate and homogenate. The reaction was started by the addition of

pyruvate. The specific activity was measured from the decrease in optical density at 340 nm.

Phosphofructokinase (E.C.2.7.1.11)

The reaction mixture contained 5 mM magnesium chloride, 200 mM potassium chloride, 50 mM Tris HCl pH 7.4, 0.1 mM NADH, 1 mM ATP, 2 mM AMP, 0.3 mM sodium cyanide, 2 mM glucose-6-phosphate, 2 IU alpha-glycerophosphate dehydrogenase and homogenate (Baldwin and Seymour 1977). The reaction was started by the addition of glucose-6-phosphate. The decrease in optical density at 340 nm was used to calculate specific activity.

NAD-linked Isocitrate Dehydrogenase (E.C.1.1.1.41)

The reaction mixture contained 20 mM phosphate buffer pH 6.5, 1.7 mM magnesium chloride, 0.17 mM manganese chloride, 0.50 mM buffered potassium cyanide, pH 7.5, 0.030 mM dichlorophenol indophenol, 0.17 mM NAD, 6.7 mM sodium DL-isocitrate and homogenate (Bennett 1972). The reaction was started by adding the isocitrate, and the decrease in optical density at 600 nm was used to calculate specific activity.

Succinic Dehydrogenase (E.C.1.3.99.1)

The reaction mixture contained 40 mM phosphate buffer, pH 7.5, 3.0 mM buffered potassium cyanide, pH 7.5, 0.040 mM dichlorophenol indophenol, 0.13 mM phenazine

methosulphate, 26 mM buffered sodium succinate, pH 7.5 and homogenate (Bennett 1972). Succinate was added to start the reaction. Activity was determined from the decrease in optical density at 600 nm.

Results

The specific activities of hexokinase, phosphorylase, lactate dehydrogenase, phosphofructokinase, NAD-linked isocitrate dehydrogenase and succinic dehydrogenase compared with those of mammalian values in Tables III-1 and III-2 (Bennett 1972, Lamb et al. 1969).

Of the four skeletal muscle glycolytic enzyme comparisons, only hexokinase showed greater activities in the mammals. Lactate dehydrogenase, phosphorylase and phosphofructokinase were greater in the reptile. However, in comparing the activities of mitochondrial enzymes, mammalian activities were greater. Reptilian hexokinase and lactate dehydrogenase activities were greater in the hepatic comparisons. Phosphofructokinase activities of the reptile were lower than those of the mammals. Both liver mitochondrial enzyme activities of the mammals were higher than the reptilian values.

Table III-1. Comparisons of anaerobic and aerobic enzyme activities between skeletal muscle of mammals and green anoles. Values taken from Crabtree and Newsholme were converted to nmoles/mg protein min assuming a ratio of 29:1 muscle tissue to muscle protein nitrogen and 108:1 liver tissue to liver protein nitrogen. Assay temperature and pH equals 33 ^{o} C and 7.4 respectively.

MUSCLE

	MAMMAL	LIZARD
Hexokinase	65.5*	0.013
Lactate Dehydronase	47300**	74200
Phosphofructokinase	238**	1051
Phosphorylase	413.8*	2522
Isocitrate Dehydrogenase	25.6**	5.6
Succinic Denydrogenase	114**	22.4

* From Crabtree and Newsholm 1972.
** From Bennett 1972.
*** From Prichard and Schofield 1968.

Table III-2. Comparisons of anaerobic and aerobic enzyme activities in liver tissue of mammals and green anoles. Values taken from Crabtree and Newsholme were converted to nmoles/mg protein min assuming a 29:1 ratio of muscle tissue to muscle protein nitrogen and a 106:1 ratio of liver tissue to liver protein nitrogen. Assay temperature and pH equals 33 ^{o} C and 7.4 respectively.

	LIVER	
	MAMMALS	LIZARDS
Hexokinase	1.2***	3.92
Lactate Dehydrogenase	16800**	27600
Phosphofructokinase	93**	56.6
Phosphorylase	--------	1058.4
Isocitrate Dehydrogenase	87.8**	24.4
Succinic Dehydrogenase	261**	73.6

* From Crabtree and Newsholme 1972.
** From Bennett 1972.
*** From Prichard and Schofield 1968

Discussion

The comparatively higher activities of glycolytic enzymes in the lizard suggest a greater capacity for anaerobic metabolism than in mammals. Likewise, the higher activities of aerobic enzymes in the mammal reflect a greater aerobic capacity. Utilizing the less efficient glycolytic pathway for energy production has the advantage of the rapid production of ATP. Other ectothermic species examined reveal the same pattern (Baldwin 1975, Baldwin and Seymour 1977, Baldwin et al. 1977, Bennett 1972, 1974, Crabtree and Newsholm 1972).

All glycolytic enzymes excluding hexokinase exhibit greater activities in skeletal muscle than in the liver. The greater activity is expected because of rapid depletion of available oxygen in skeletal muscle during activity and subsequent anaerobiosis.

Phosphorylase activity in skeletal muscle is approximately 2.5 times higher than its activity in liver. Phosphorylase in its active form, phosphorylase-a (Figure III-3), catalyzes the breakdown of glycogen at a very high rate to glucose-1-phosphate. Glucose-1-phosphate can then be shuttled through glycolysis after isomerization into glucose 6-phosphate. This mobilization of glucose-1-phosphate from glycogen is very important in the liver and skeletal muscle. Under most physiological conditions

phosphorylase-b is inactive because of the inhibitory effects of ATP and glucose-6-phosphate (Stryer 1981). The greater activity of phosphorylase in skeletal muscle may be due to the speed at which the end product is used. Phosphorylase is the control point in an amplification cascade in hepatic and extrahepatic tissues (Figure III-4). In liver tissue the end result of this cascade is the release of free glucose into the bloodstream. Skeletal muscle lacks glucose-6-phosphatase and cannot produce free glucose. Thus all glycogen stored in skeletal muscle is only used by muscle: skeletal muscle does not act as a glycogen reserve for other tissues.

Several minutes may be required for glycogen phosphorylase to reach peak activity in the liver (Lehninger 1982). Because of the inherent amplification involved with each reaction step, hepatic phosphorylase may possess lower activity because of the tremendous production from each subsequent step in the cascade. The amplification is estimated to be some 25 million fold. The cascade in skeletal muscle is lower and, therefore, the amplification is reduced. In addition, under certain conditions, the energy required by the muscles must be instantaneous. In situations where rapid escape behavior or prey ambush is required, any delay in glucose utilization may be deleterious.

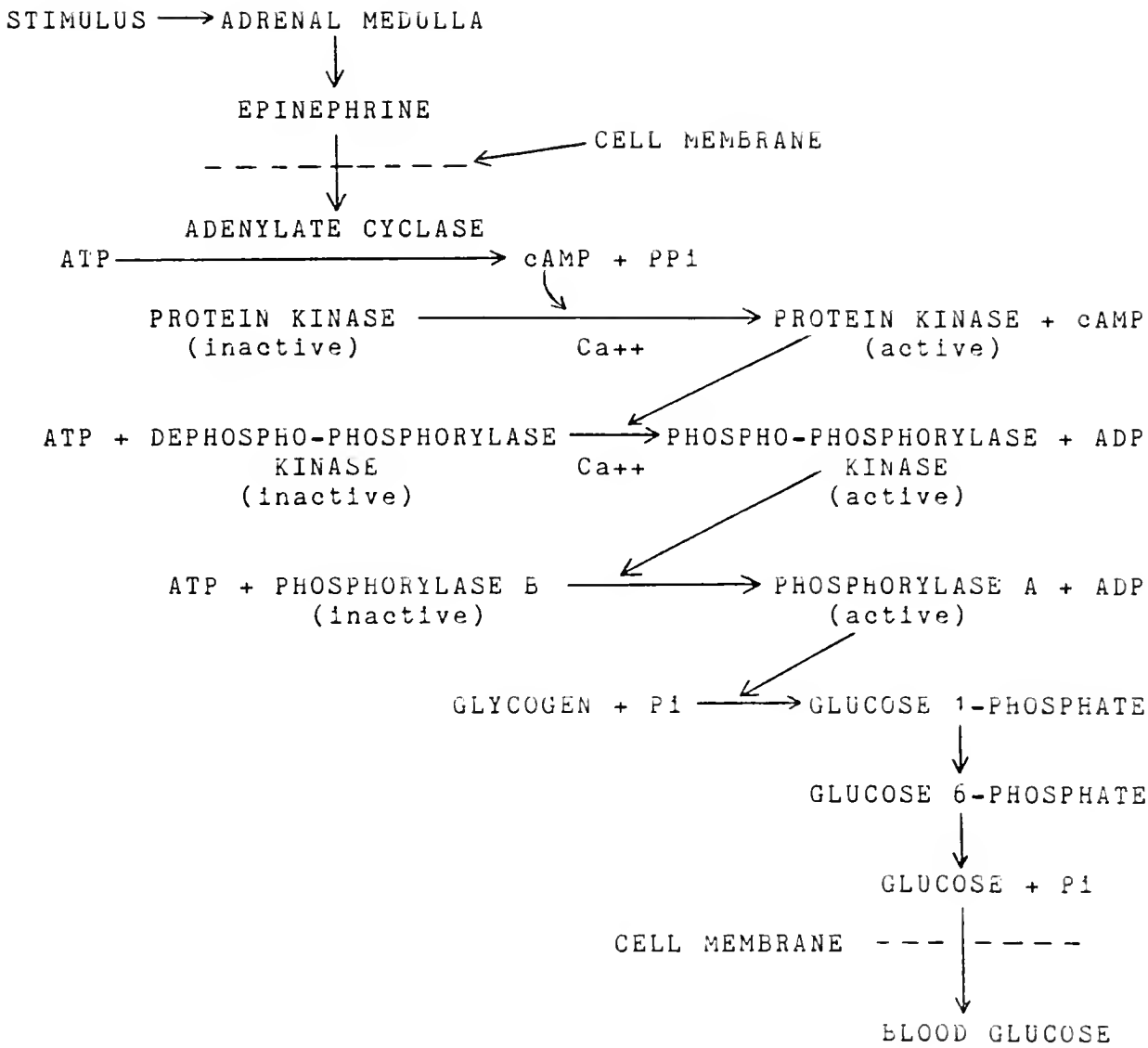


Figure III-4. Epinephrine produced amplification cascade in hepatic cells. Epinephrine arrives at the receptor sites on the cell membrane at a concentration of approximately one nM. The free glucose released into the blood increases its concentration by 5 mM. The multiplier effect produced is about 3 milllion times.

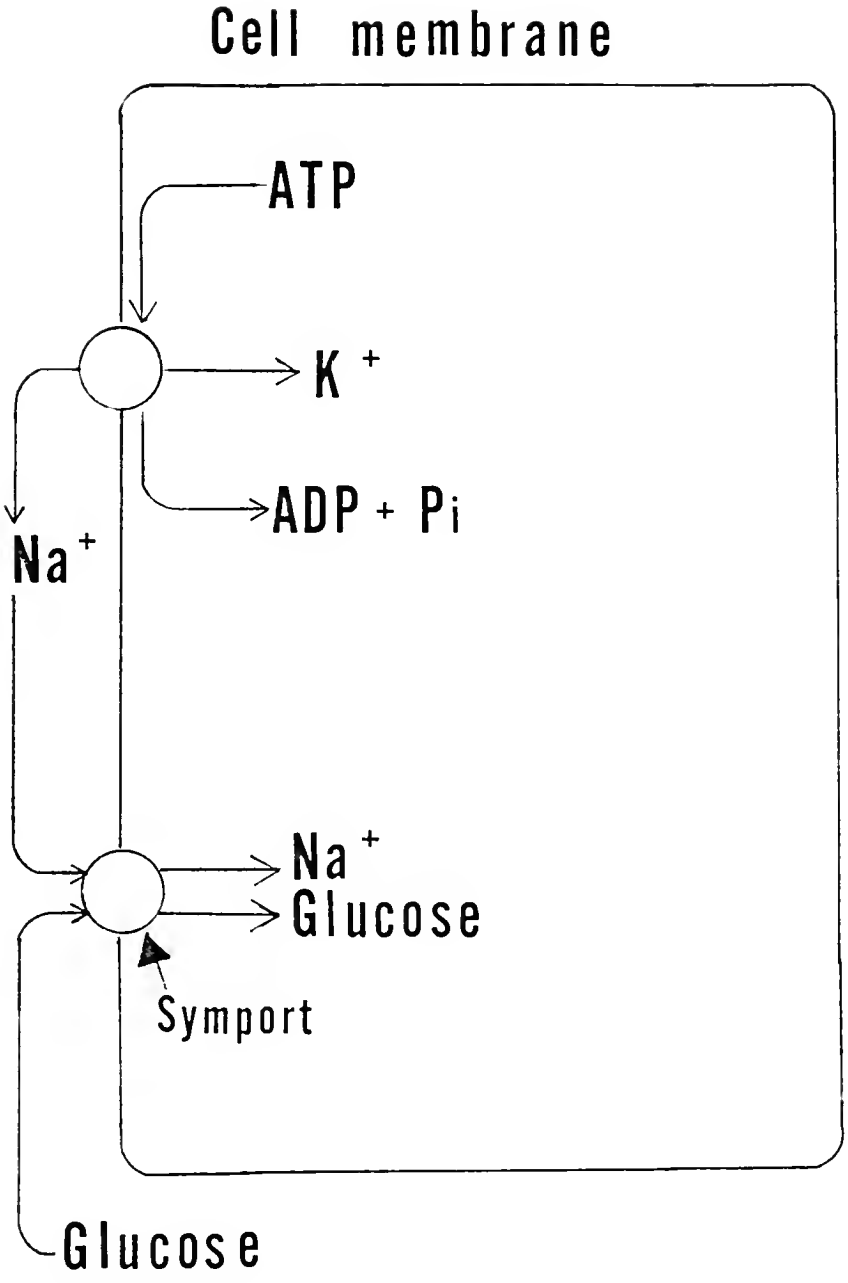
Low hexokinase activities in skeletal muscle may reflect the inability of this tissue to use free glucose from the blood as a readily available energy source. Low hexokinase activities have been reported for other poikilotherms (Baldwin and Seymour 1977, Baldwin et al. 1977, Bennett 1972, 1974, Crabtree and Newsholm 1972). Enzymes with low specific activities that form part of the chain of a metabolic pathway usually catalyze non-equilibrium reactions (Newsholme and Start 1973). This is indicative of fewer active sites in the enzyme molecule than is required to reach equilibrium. The greater catalytic capacities of preceeding and subsequent enzymes form substrates and remove products faster than the non-equilibrium enzyme can inter-convert them. Thus, enzymes that control non-equilibrium reactions form a bottleneck in metabolic pathways. Hexokinase, phosphofructokinase and phosphorylase are prime candidates for catalyzing non-equilibrium reactions.

By comparing mass-action ratios (t) with apparent equilibrium constants (K') a reaction can be evaluated as being non-equilibrium or not. If the value of t is much smaller than K', the reaction is displaced far from equilibrium. Comparisons between t and K' in mammalian tissues show hexokinase, phosphofructokinase and phosphorylase all catalyze non-equilibrium reactions (Newsholm and Start 1973). Similar results were also found in spiders (Prestwich 1982).

Mass action ratios were not measured here. However, the similar enzyme activities found here and in studies of other ectotherms, indicate that very few if any differences in the glycolytic pathway exists (Baldwin and Seymour 1977, Baldwin et al. 1977, Bennett 1972, Macleod et al. 1963). If no differences exist in the descriptive aspects of the pathway, the same bottlenecks will occur at the same locations. However, the higher activities of certain key steps in poikilotherms, enhance the output of this pathway.

In emergency situations or during periods of fasting the liver, mobilizing glycogen stores, releases free glucose into the blood. Glucose molecules enter cells by means of a symport (Figure III-5). Once inside the cell glucose is phosphorylated by hexokinase at the expense of one ATP. By phosphorylating all the glucose that enters the cell, a large glucose concentration gradient is maintained between the blood and the intracellular compartment. Low hexokinase activity reduces this potential gradient through the reduction of glucose-6-phosphate formation. One potential solution to low hexokinase activity is maintaining high blood glucose levels. High blood glucose levels may serve to continously flood the intracellular environment with glucose. Maintaining high levels of glucose intracellularly may allow the low levels of hexokinase to phosphorylate large numbers of glucose molecules. This is discussed further in Chapter IV.

Figure III-5. The glucose sodium symport. The sodium gradient produced powers the transport of glucose. Adapted from Stryer 1981.


Cell membrane
ATP
K +
ADP + Pi
Na +
Na +
Glucose
Symport
Glucose

Seasonal variation may be involved in enzyme activities, as may the rate of metabolism. All enzyme assays were performed on tissue samples from resting animals during late summer when plasma glucose levels are declining and tissue glycogen levels are low. During this time enzymes are expected to be most effective. If true, muscle hexokinase activities would be even more reduced during times when glucose and glycogen levels are high. Ozand and Narahara (1964) reported increases in the phosphofructokinase reaction rate in contracting muscle. They suggested that under conditions in which the phosphofructokinase of the muscle is saturated with fructose-6-phosphate, an increase in Vmax of the reaction may involved. If we assume that regulatory enzyme function follows a conservative pattern, hexokinase activity may also show an increase with an increase in metabolism.

The nigher activities of the mitochondrial enzymes in the mammal may be due to either or both of two reasons. First it may be simply due to a more rapid conversion of substrate with no difference in the concentration of enzyme between the anole and the rat. However the amount of enzyme present in the mammalian tissue may be greater due to either a greater proportion of mitochondria, greater relative membrane surface area, or both. In comparing the activity of cytochrome oxidase in four

different tissues of a reptile and a mammal, Else and Hulbert (1981) reported higher activities in the latter in all four tissues. They attributed this to greater mitochondrial volume densities and mitichondrial membrane surface areas in the mammal. A greater mitochondrial volume density in mammals is probably related to the use of fatty acids as a metabolic substrate (Bennett 1972, Drummond 1971). Because of the preferential use of glucose as an energy substrate for activity in reptiles, fatty acid metabolism is only utilized during specific times such as fasting and reproduction (Dessauer 1955, Smith 1968).

CHAPTER IV
THE EFFECTS OF ACTIVITY AND FASTING ON TISSUE GLYCOGEN AND PLASMA GLUCOSE LEVELS

Introduction

Poikilothermic ectotherms are generally regarded as low energy systems. This is especially true when they are compared metabolically to endothermic homeotherms (Bennett 1972). Reptiles generally are not as active as are mammals or birds. The basis for this less active lifestyle is the preferential use of anaerobic, glycolytic pathways instead of aerobiosis during intense activity. More than 50% of the total energy produced from activity is derived from anaerobiosis (Bennett and Licht 1972, Gleeson 1982, Gratz and Hutchison 1977, Pough and Andrews 1983). Although glycolysis is less efficient than aerobiosis (i.e., Krep's cycle), the former produces ATP's at a much faster rate.

Reliance on glycolysis for energy production suggests that glucose is a limiting factor in metabolism during activity in poikilotherms. Muscle glycogen depletion occurs in most vertebrates after intense activity (Dean and Goodnight 1964, Gaesser and Brooks 1980, Gleeson

1982, Gratz and Hutchison 1977, Johnston and Goldspink 1973, MacDougall et al. 1977, Piehl 1974). During the recovery period following depletion of muscle glycogen, small reptiles are particularly susceptible to predation. Their susceptibility increases with the time course of glycogen replenishment.

This chapter examines the effects of burst activity on tissue glycogen, blood glucose, lactate production and the effects of fasting on carbohydrate utilization before, during and after exercise. Additionally the relationship between carbohydrate loading and glycogen replacement is also evaluated.

Methods

Glucose and glycogen analyses were performed during July and August 1986, as described in Chapter II. Animals were exercised in a water-driven treadmill at their eccritic temperature (33 ^{o} C), until exhausted. Exhaustion is defined as loss of the righting response. Immediately upon exhaustion the animals were either dropped into liquid nitrogen and assayed for tissue glycogen and lactate or decapitated and the blood assayed for glucose or lactate. Glucose and glycogen concentrations were measured at rest, 30 seconds after the onset of exercise, at the point of exhaustion and at

various times up to 720 minutes during recovery. Recovery here and in all subsequent assays is defined as the time from the end of exercise to when pre-exercise (resting) levels of the parameter in question are reached. Plasma glucose is expressed as mg/dl. Tissue glycogen is expressed as mg glycogen/g of tissue. All animals were maintained in a photothermal gradient (22 ^{o}C to 50 ^{o}C, 12:12 light:dark cycle). All means are expressed with their standard errors.

Lactate Analysis

Ten groups of five animals each were analyzed at rest, after exhaustion, or 10, 60 or 120 minutes during the recovery period. One half of the groups were used to analyze whole body lactate concentrations. Lizards were decapitated and dropped into liquid nitrogen. After freezing, the bodies were homogenized in volumes of cold 0.6 N perchloric acid equivalent to 12 times body mass.

To study compartmentilization of lactate, samples of blood, liver and leg muscle from the animals of the other five groups were weighed, pooled in cold perchloric acid (5 times the sample weight) and homogenized. All homogenates were centrifuged at 3000 rpms at 4 ^{o}C for 10 minutes. The supernatant was filtered and recentrifuged at 10000 rpms at 4 ^{o}C for 10 minutes. The resulting supernatant was assayed with an enzymatic test kit (Sigma

Chemical Company). Samples were read on a model 6/20 Coleman Junior 2 spectrophotometer at 340 nm. Lactate concentrations are expressed as mg lactate/g tissue weight.

Oxygen Consumption

Oxygen consumption ($\dot{V}_{O2}$) was measured manometrically using a Gilson Differential Respirometer during July and August 1986. Lizards (n = 5) were placed in 125 ml respirometer flasks. Each flask contained approximately 12 grams of soda lime to absorb CO_2. All determinations were made at 33 ^{o}C (i.e., eccritic temperature). Resting rates were measured at 10:00 p.m. E.S.T.

After several hours of acclimation in the flasks $\dot{V}_{O2}$'s were measured. To measure oxygen consumption after exhaustion and during recovery, animals were exercised at 9:00 p.m. in the respirometry flasks by the motorized shaking of the flasks. To increase the activity of the lizards in the flasks, small glass spheres were added. The movement of the spheres agitated the lizards thereby causing activity. During the recovery period rates were measured every 2 minutes. Rates are expressed as ml O_2/g·h.

Carbohydrate Loading

The experiments were performed in August, the time when body sugar levels are lowest. The role of these experiments was to explore the relationship between glucose supplementation and glycogen resynthesis. Lizards were fasted for 48 hours (n = 120). After exercising to exhaustion, half the lizards were fed a glucose solution, 20 ul/g body weight at a concentration of 1 mg/4 ul by stomach tube and allowed to recover for 12 hours. This volume and concentration is equivalent to 5 mg glucose per g body weight. The other lizards were given an equivalent volume of distilled water via a stomach tube. After 12 hours one half of the glucose supplemented lizards were assayed for tissue glycogen as previously described. One half of the control group were assayed for tissue glycogen. The other glucose supplemented lizards were given a second glucose feeding and were assayed 12 and 24 hours later. The rest of the control lizards were also assayed at these times.

Fasting

These experiments examined the effect of fasting on glucose concentrations at rest, during exercise and during recovery. Lizards fasted for 2 weeks during August. After fasting the animals were exercised as previously described until exhaustion. Glucose and glycogen levels were measured at the same intervals as previously described.

Results

Oxygen consumption and whole body lactate at rest, during exercise and recovery are summarized in Figure IV-1. Maximum $\dot{V}_{O2}$ occurred during exercise (1.65 ± 0.201 ml/g·h). With a resting $\dot{V}_{O2}$ of 0.262 ± 0.038 ml/g·h, the aerobic scope equals 6.3. Oxygen consumption declined steadily throughout the recovery period. At 75 minutes of recovery no statistically significant difference existed in the recovery and resting rates of oxygen consumption ($P > 0.05$, T-test for paired data). Table IV-1 summarizes the net recovery oxygen consumption and the change in lactate concentration during recovery.

Whole body lactate increased 8.4 times from resting values, 0.29 ± 0.01 mg/g, to exercise values 2.43 ± 0.21 mg/g. Over the first 10 minutes of recovery lactate declined very little (2.36 ± 0.26 mg/g). Sixty minutes into recovery, lactate had dropped drastically (0.68 ± 0.14 mg/g). By 120 minutes the lactate levels were not statistically different from those of resting values ($P > 0.05$, paired T-test).

Figure IV-2 summarizes the data for lactate compartmentalization. All compartments show the same trend. Maximal lactate concentrations occur during exercise. Muscle and blood lactate levels after 2 hours of recovery were

Figure IV-1. Lactate concentration and oxygen comsumption before, during and after exercise. Circles = oxygen consumption. Triangles = lactate. Each symbol represents the mean of 5 lizards.

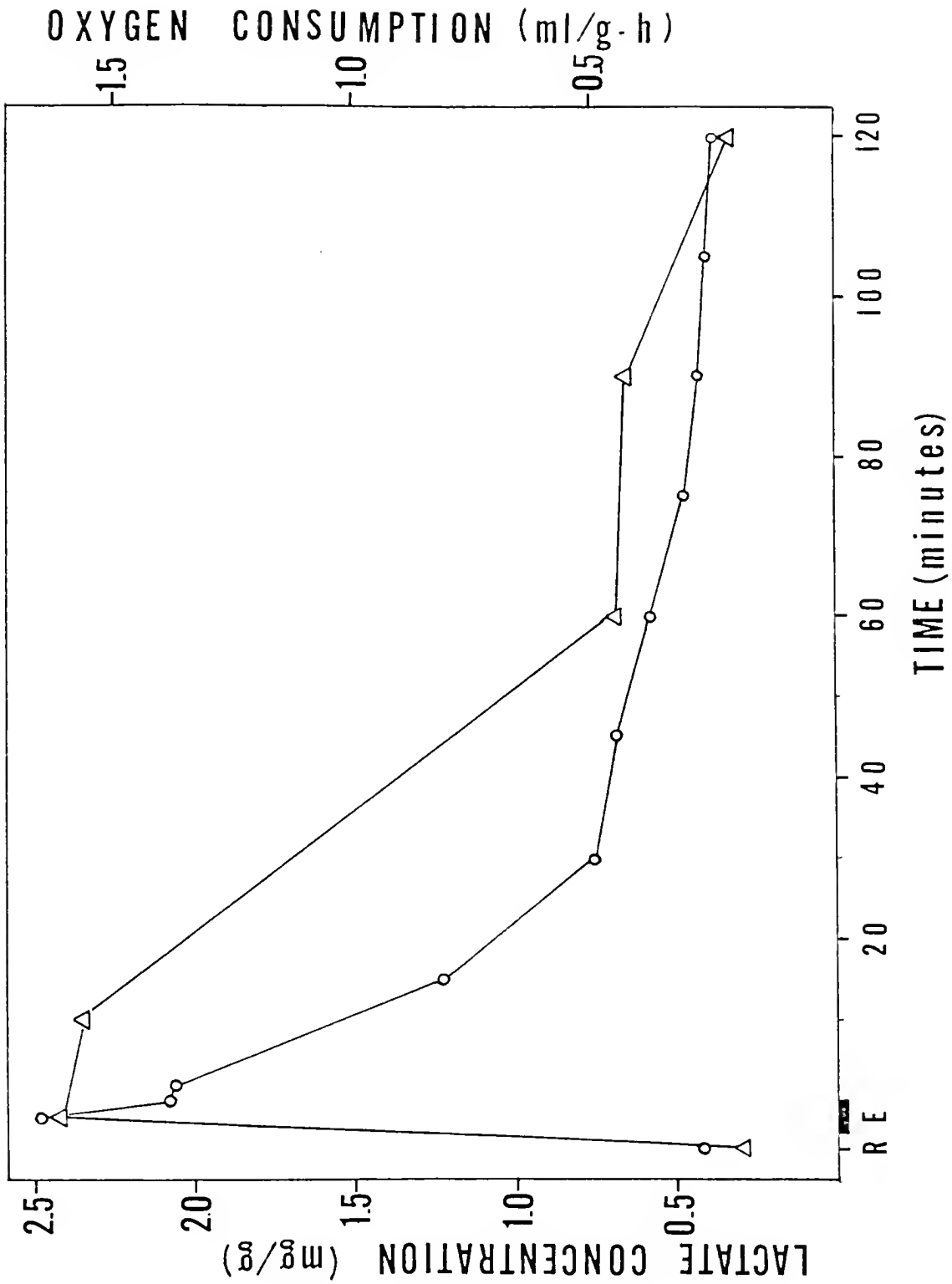
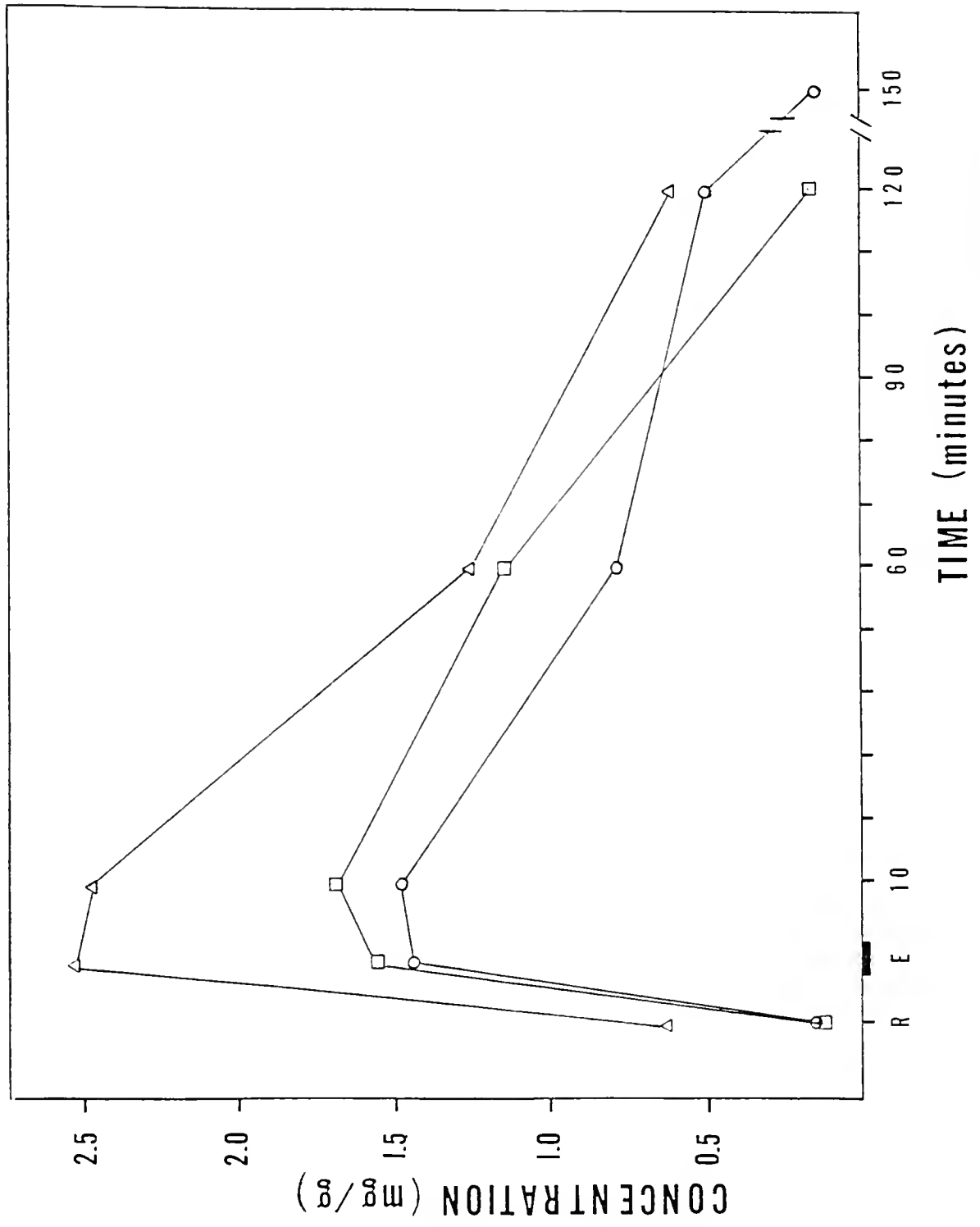

OXYGEN CONSUMPTION (ml/g·h)
1.5
1.0
0.5
LACTATE CONCENTRATION (mg/g)
2.5
2.0
1.5
1.0
0.5
R E
20
40
60
80
100
120
TIME (minutes)

Table IV-1. Resting and post exercise lactate and glycogen levels. Units are mg lactate/glycogen per g tissue.

	NONFASTING		FASTING	
Muscle	REST	POSTEXERCISE	REST	POSTEXERCISE
Lactate	0.62	2.53	----	----
Glycogen	4.1	1.6	3.9	1.9
Liver				
Lactate	0.15	1.44	----	----
Glycogen	7.1	3.9	5.4	2.3

Figure IV-2. The compartmentalization of lactate before, during and after exercise. Squares = plasma lactate. Triangles = skeletal muscle lactate. Circles = liver lactate. Each symbol represents the mean of 5 lizards.


CONCENTRATION (mg/g)
2.5
2.0
1.5
1.0
0.5
R E 10
60
90
120
150
TIME (minutes)

statistically indistinguishable from resting values ($P > 0.05$, T-test). Liver lactate levels during recovery were the same as resting levels by 2.5 hours. Resting muscle lactate levels were significantly greater than those of the liver and blood ($P > 0.05$, T-test). Resting liver and blood lactate levels are very similar (0.15 mg/g and 0.12 mg/g respectively).

Muscle glycogen and lactate levels show the expected trends (Figure IV-3). As exercise proceeds glycogen levels drop and lactate concentrations increase. As activity ceases lactate begins to decrease with a concomitant increase in muscle glycogen. At 120 minutes into recovery lactate levels are not statistically different from resting levels (0.59 ± 0.07 mg/g and 0.62 ± 0.05 mg/g, respectively, $P > 0.05$, T-test). Muscle glycogen returns to resting levels 6 h after the cessation of activity (4.3 ± 0.17 mg/g and 4.1 ± 0.33 mg/g, respectively, $P > 0.05$, T-test).

Liver glycogen and lactate concentrations also show opposite changes (Figure IV-4). Liver glycogen levels do not drop significantly until the end of exercise. No significant change in liver glycogen occurs after one minute into exercise (7.1 ± 0.83 mg/g and 7.0 ± 0.87 mg/g respectively, $P > 0.05$, T-test). At the end of activity, the glycogen level drops to 3.9 ± 0.44 mg/g. Liver lactate increased from a resting level of 0.15 ± 0.02 mg/g to

Figure IV-3. Muscle glycogen and lactate before, during and after exercise. Circles = glycogen. Triangles = lactate. Each symbol represents the mean of 5 lizards. The vertical bars are one standard error.

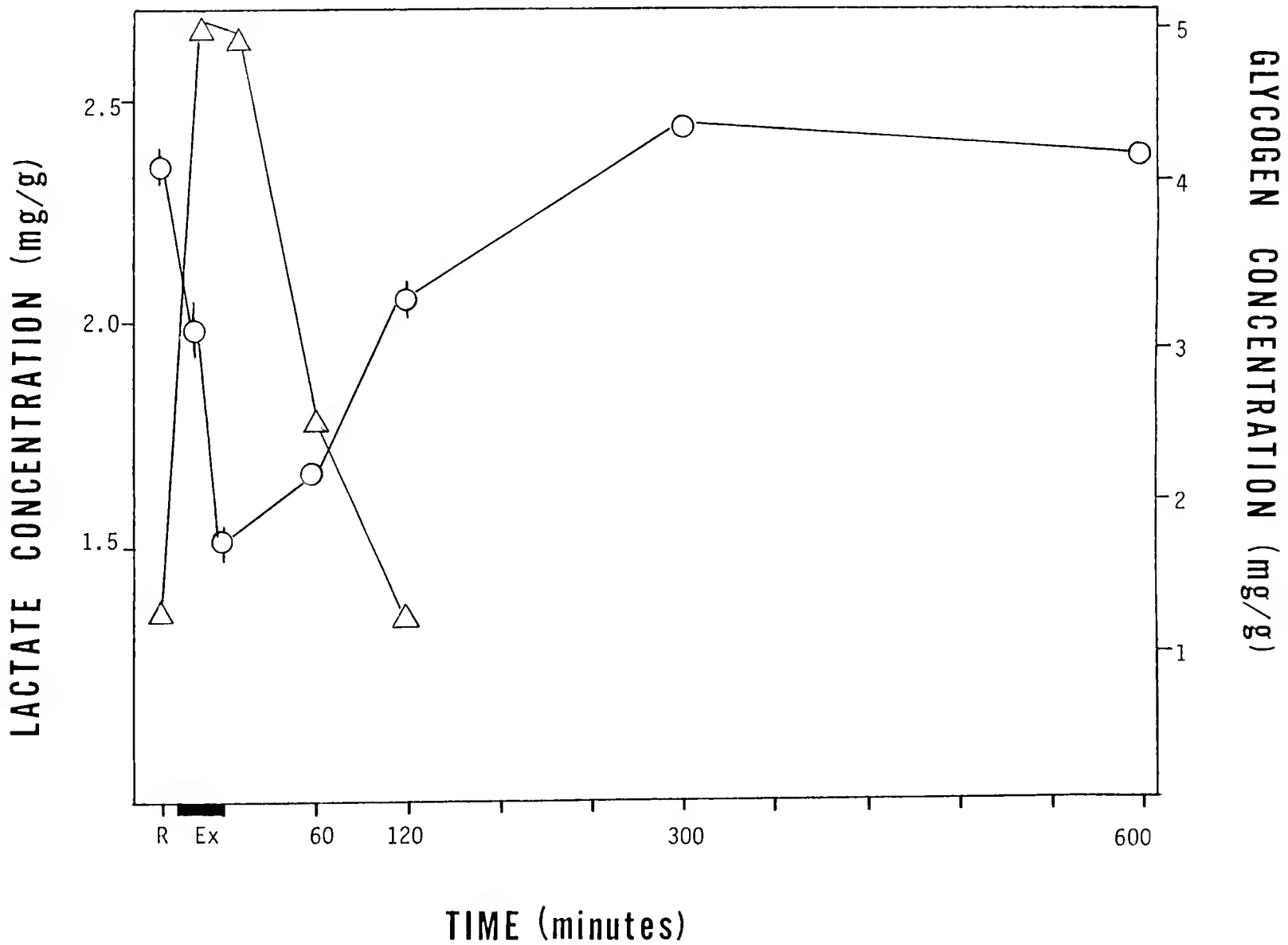
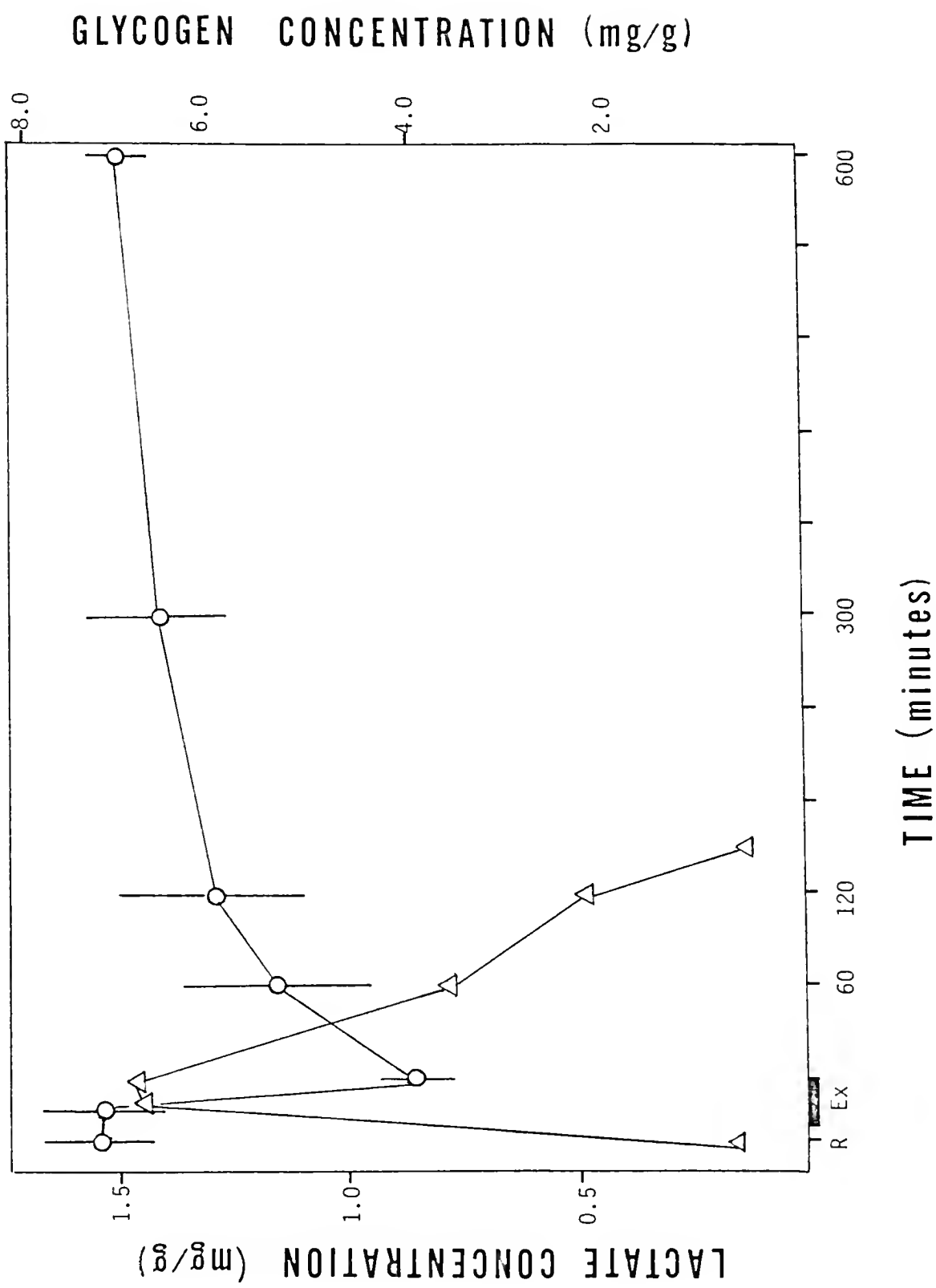

LACTATE CONCENTRATION (mg/g)
2.5
2.0
1.5
GLYCOGEN CONCENTRATION (mg/g)
5
4
3
2
1
R
Ex
60
120
300
600
TIME (minutes)

Figure IV-4. Liver lactate and glycogen before, during and after exercise. Triangles = lactate. Circles = glycogen. Each symbol represents the mean of 5 lizards.


GLYCOGEN CONCENTRATION (mg/g)
8.0
6.0
4.0
2.0
LACTATE CONCENTRATION (mg/g)
1.5
1.0
0.5
R
Ex
60
120
300
600
TIME (minutes)

1.44 ± 0.11 mg/g at the end of exercise. Lactate levels continue to rise after exercise reaching a peak value of 1.47 ± 0.14 mg/g after 10 minutes of recovery. Lactate levels then declined to 0.78 ± 0.03 mg/g after 60 minutes of recovery. After 2.5 hours of recovery lactate levels returned to resting levels (0.13 ± 0.008 mg/g and 0.15 ± 0.02 mg/g respectively, $P > 0.05$, T-test). Resting and peak lactate and glycogen concentrations in muscle and liver with time to recovery are summarized in Table IV-2.

Plasma glucose concentrations before, during and after exercise are summarized in Figure IV-5. Plasma glucose in the post-exercise period reached higher levels than pre-exercise levels. During exercise plasma glucose values fell from 179.6 ± 14.3 mg/dl to 149.6 ± 12.3 mg/dl. The plasma glucose level reaches its maximum concentration, 239.4 ± 12.1 mg/dl two hours into recovery. This level remains fairly constant for the next three hours. After 12 hours, plasma glucose levels decline to 134.1 ± 6.7 mg/dl.

Carbohydrate loading during recovery produced different results for muscle and liver glycogen replenishment. The initial glucose supplement had no effect on muscle glycogen replenishment (Figure IV-6). After 12 hours control and experimental values were not different (4.5 ± 0.07 mg/g and 4.6 ± 0.04 mg/g, $P > 0.05$, T-test). Compensation

Table IV-2. Oxygen consumption requirements for lactate oxidation and gluconeogenesis from lactate. Numbers are based on data taken from Figure IV-2.

TIME (MIN)	LACTATE (mM)	NET OXYGEN RECOVERY CONSUMPTION (ml)	OXYGEN REQUIRED (ml) LACTATE TO GLUCOSE 6-P	LACTATE OXIDATION
0-4	0.072	0.098	7.6×10^{-7}	4.838
4-15	0.070	0.074	7.4×10^{-7}	4.704
15-45	0.020	0.029	2.0×10^{-7}	1.344
45-75	0.019	0.010	2.0×10^{-7}	1.277
75-120	0.009	0.002	9.5×10^{-9}	0.605

Figure IV-5. Plasma glucose concentration before, during and after exercise. Triangles = fasting animals. Squares = nonfasting animals. Each symbol represents the mean of 5 lizards. The vertical bars are one standard error.

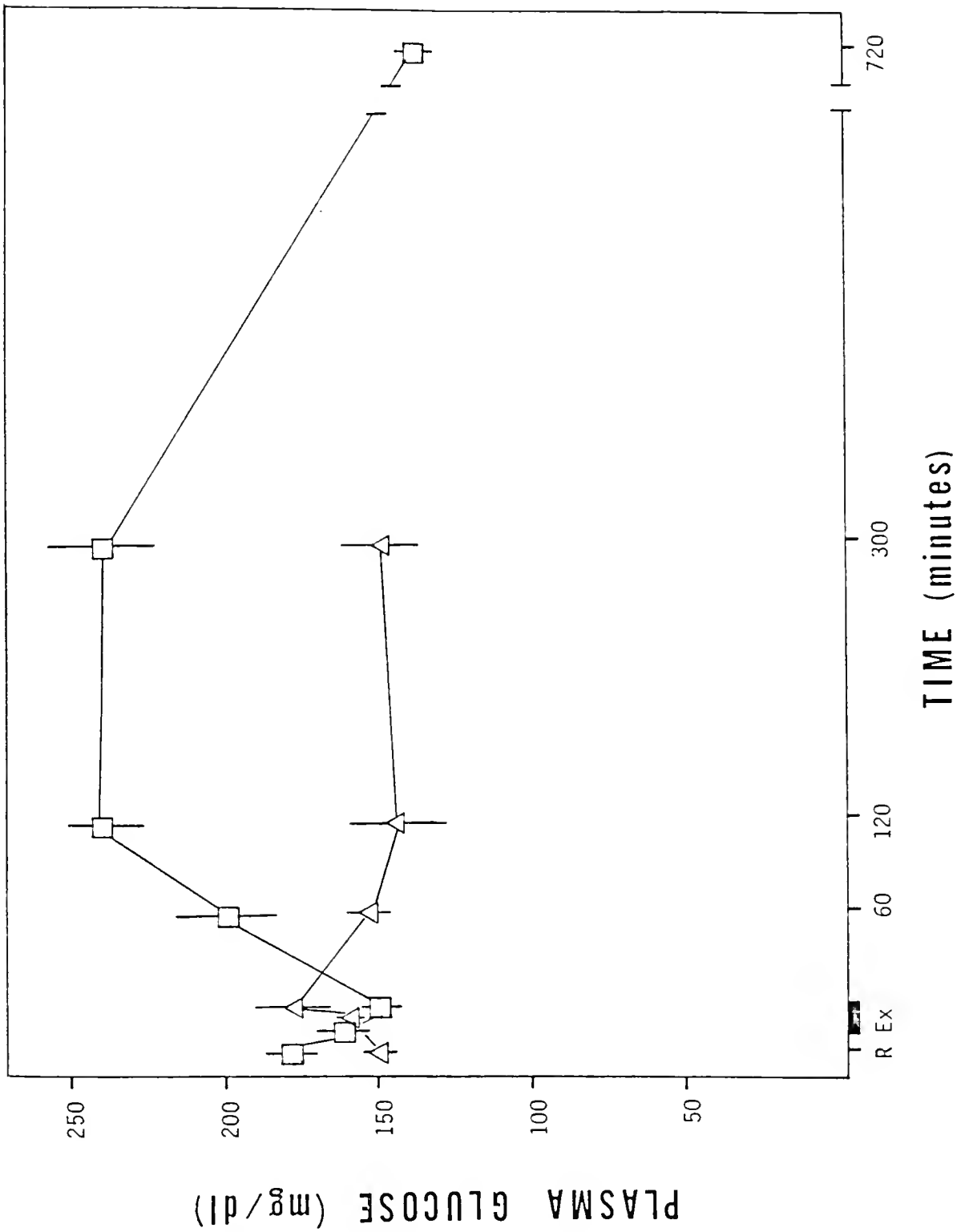
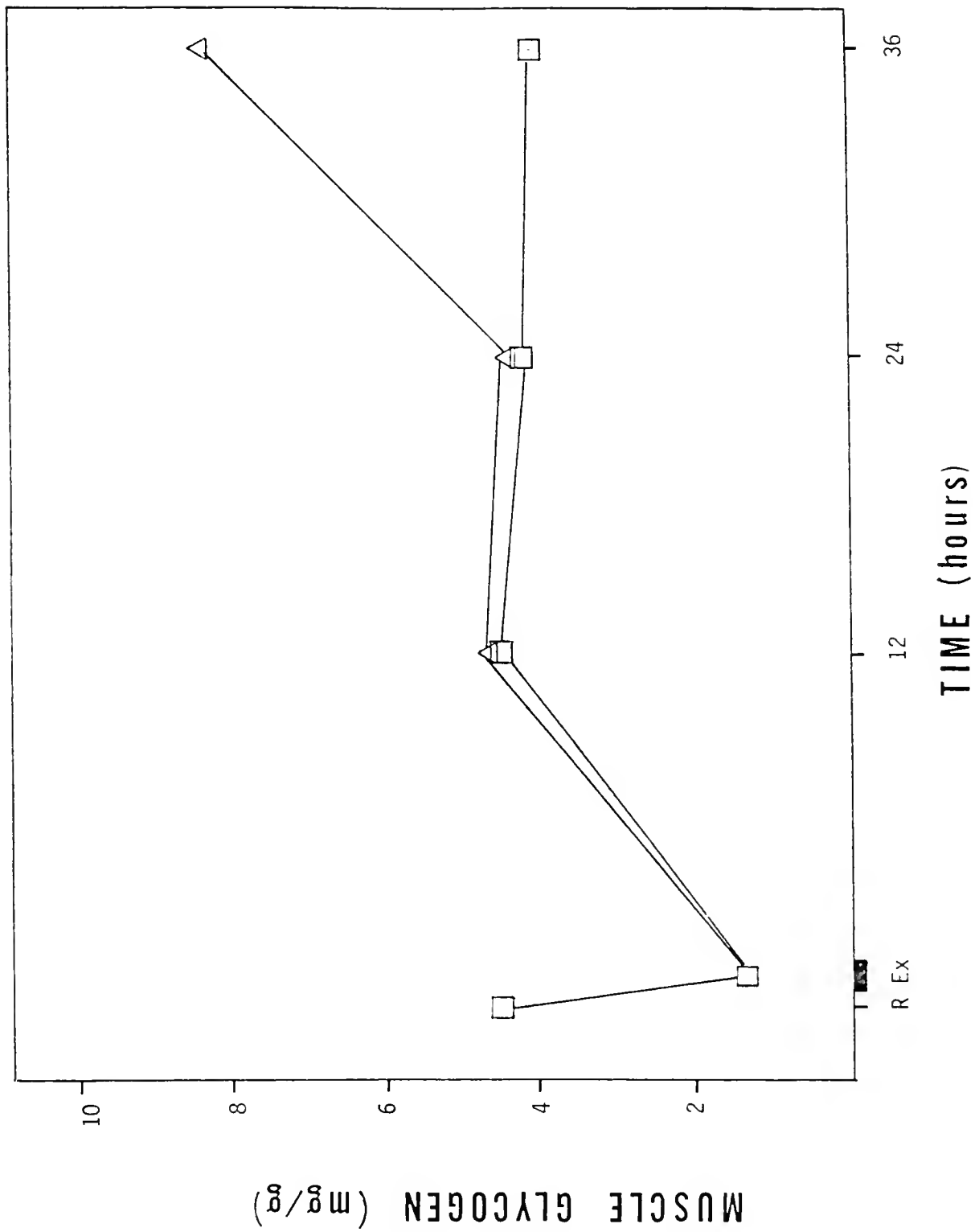

PLASMA GLUCOSE (mg/dl)
250
200
150
100
50
R Ex
60
120
300
720
TIME (minutes)

Figure IV-6. Effects of post exercise carbohydrate loading on muscle glyocgen. Squares = controls. Triangles = animals receiving glucose load. N = 120. The vertical bars are one standard error. Glucose is given at the end of exercise.


MUSCLE GLYCOGEN (mg/g)
10
8
6
4
2
R Ex
12
24
36
TIME (hours)

occurred only in muscle after the second glucose feeding. After the second feeding muscle glycogen levels increased almost 2 times (4.6 ± 0.04 mg/g to 8.4 ± 0.23 mg/g. Overcompensation occurred after both glucose feedings in liver tissue (Figure IV-7). Liver glycogen had increased 10 times over resting levels after the second feeding (7.0 ± 0.66 mg/g to 70.1 ± 4.08 mg/g).

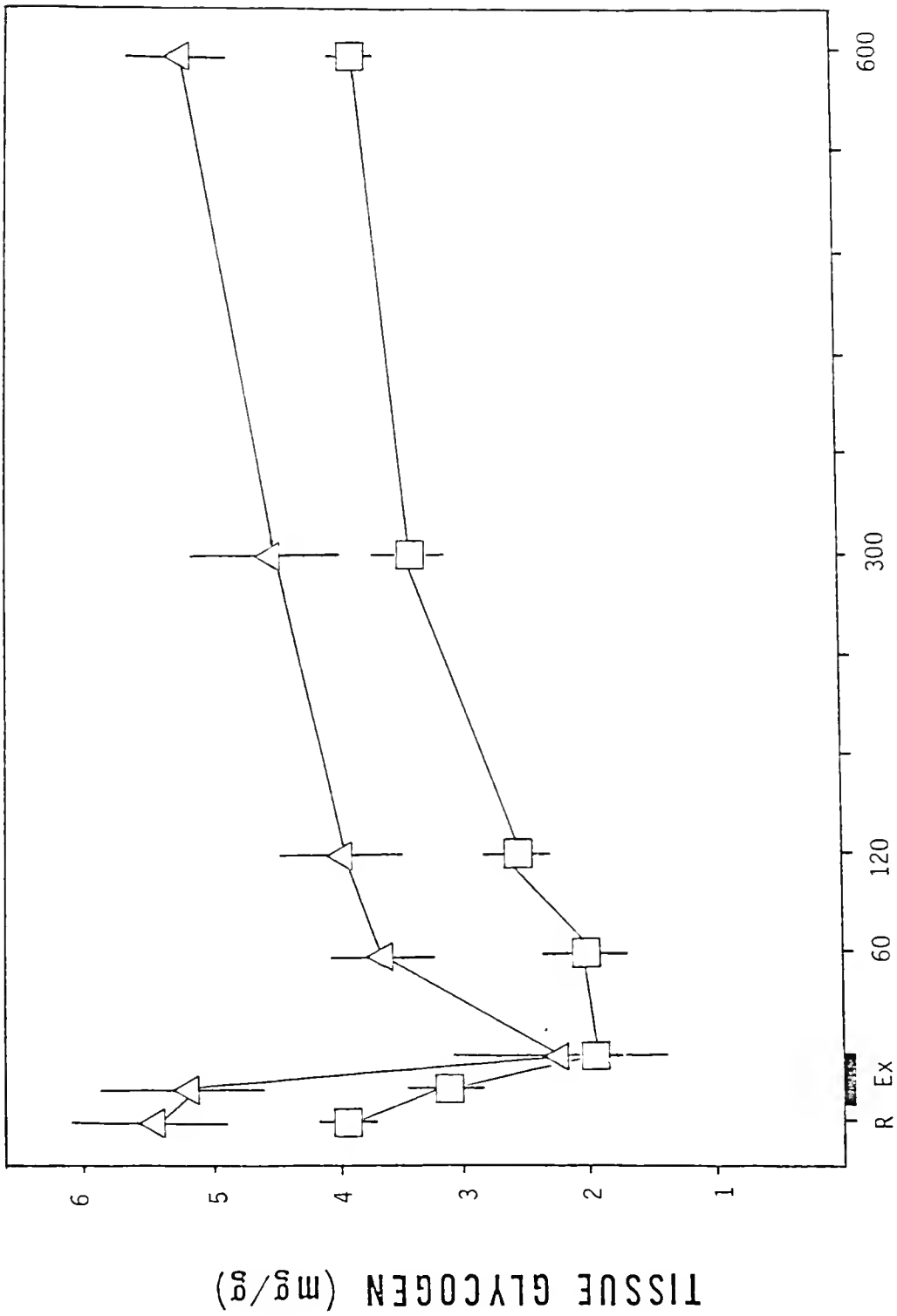
Plasma glucose is affected differently during exercise in fasting and nonfasting animals (Figure IV-5). Plasma glucose of fasting lizards increased from 149.6 ± 13.1 mg/dl to 177.1 ± 18.1 mg/dl. Glucose levels declined rapidly, and after 60 minutes of recovery, plasma levels were equal to resting levels (151.2 ± 10.3 mg/dl and 149.6 ± 13.1 mg/dl respectively, $P > 0.05$, T-test). Plasma levels then dropped and remained slightly below resting levels.

Resting tissue glycogen concentrations are lower in fasting animals than nonfasting animals (Figure IV-8). The same patterns during and after activity are produced among fasted lizards as seen among nonfasted individuals (Figures IV-3, IV-4). However glycogen levels are generally depressed among fasted animals. Peak resting and recovered muscle and liver glycogen values are summarized in Table IV-1.

Figure IV-7. Effects of post exercise carbohydrate loading on liver glycogen. Squares = controls. Triangles = animals receiving glucose load. N = 120. The vertical bars are one standard error. Glucose is given at the end of exercise.

LIVER GLYCOGEN (mg/g)
80
70
60
50
40
30
20
10
R
Ex
12
24
36
TIME (hours)

Figure IV-8. Tissue glycogen before, during and after exercise in fasted lizards. Squares = muscle glycogen. Triangles = liver glycogen. Each symbol represents the mean of 5 lizards. The vertical bars are one standard error.


TISSUE GLYCOGEN (mg/g)
6
5
4
3
2
1
R Ex
60
120
300
600
TIME (minutes)

Discussion

A characteristic prolonged elevation of oxygen consumption occurs after exercise in vertebrates. Hill et al. (1924) originally termed this the oxygen debt. This elevated oxygen consumption was interpreted as the oxidation of lactic acid produced as the result of tissue glycogen catabolism. The original hypothesis stated that only a fraction of the lactate, approximately 20%, was actually oxidized. The lactate oxidized provided the energy required to resynthesize glycogen from the remaining lactate. Results from several studies have questioned the validity of this hypothesis (Bennett and Licht 1973, Brooks et al. 1971, 1973, Gleeson 1980a, 1980b, Gratz and Hutchison 1977, Hutchison et al. 1977). Results of this study also appear to refute the classic oxygen debt hypothesis.

Seventy-five minutes into the post-exercise recovery period oxygen consumption has returned to resting values (Figure IV-1). Post exercise lactate levels require two hours to return to resting levels. These data show that net lactate removal is still occurring after oxygen consumption has returned to normal. However, muscle and liver glycogen levels return to pre-exercise levels without any carbohydrate intake. The time course for this tissue glycogen replenishment is several hours (Figures IV-3, IV-4).

Lactate removal appears, in Figure IV-1, not to be the result of elevated post-exercise oxygen consumption (EPOC). However, the temporal uncoupling between lactate removal and EPOC is misleading. The metabolic pathway(s) used for lactate removal cannot be determined without using radio-labeled compounds. The fate of lactate can be suggested by calculating the amount of oxygen required to resynthesize glucose from lactate (i.e., gluconeogenesis), or to completely oxidize lactate to carbon dioxide and water (Appendix), and by comparing these estimates with those actually measured. Table IV-2 is such a summary. Obviously the increase in oxygen consumption during recovery cannot supply the energy required to completely oxidize lactate (Table IV-2). The net recovery oxygen consumption only accounts for approximately 0.3 - 2% of the energy required for complete lactate oxidation. However, the net recovery oxygen consumptionis more than adequate for the resynthesis of glucose from lactate.

These comparisons suggest that most if not all of the lactate produced during forced activity is reconverted into glycogen via gluconeogenesis. This differs from the situation in mammals, in which the primary fate of lactate is oxidation (Brooks et al. 1973, Drury and Wick 1956, Gaesser and Brooks 1980). The production of glycogen from lactate appears to occur in other reptiles (Gleeson 1985, Moberly 1968). Gleeson (1985), using

Table IV-2. Oxygen consumption requirements for lactate oxidation and gluconeogenesis from lactate. Numbers are based on data taken from Figure IV-2.

TIME (MIN)	LACTATE (mM)	NET OXYGEN RECOVERY CONSUMPTION (ml)	OXYGEN REQUIRED (ml) LACTATE TO GLUCOSE 6-P	LACTATE OXIDATION
0-4	0.072	0.098	7.6×10^{-7}	4.838
4-15	0.070	0.074	7.4×10^{-7}	4.704
15-45	0.020	0.029	2.0×10^{-7}	1.344
45-75	0.019	0.010	2.0×10^{-7}	1.277
75-120	0.009	0.002	9.5×10^{-9}	0.605

inferential data reported seasonal variation in the ability of red muscle to produce glycogen from lactate. He suggested that muscle gluconeogenesis may account for approximately 20% of the lactate removed during recovery from exhaustive activity. because many reptiles rely on burst activity in many of their behaviors, utilizing the gluconeogenic pathway for lactate removal allows them to resynthesize the much needed substrate for its reuse.

The compartmentalization of lactate also suggests the possibility of a gluconeogenic fate for lactate (Figure IV-2). Liver lactate levels during recovery remain elevated after blood and muscle lactate levels have returned to pre-activity levels, which suggests that the liver may be responsible for the reduction of lactate levels. The liver has been shown to possess relatively high specific activities of lactate dehydrogenase (Chapter III), an enzyme that is required to convert lactate back into pyruvate before it is metabolized. This conversion is necessary to regenerate NAD+ so the glycolytic pathway remains active as long as possible. As such the metabolic burden of energy production does not fall on the skeletal muscle alone. Part is shifted from muscle to the liver (Stryer 1981).

The potential for the liver as the primary site of gluconeogenesis is strengthened when one examines the plasma glucose levels during recovery (Figure IV-5).

Plasma glucose levels remain elevated for at least 5 hours following forced activity, which may be due to the reconversion of lactate to glucose by the liver. After the conversion of lactate to glucose by the liver, free glucose enters the bloodstream from which it is stored by various tissues as glycogen. Glycogen then serves as the primary substrate for catabolic energy production. Plasma glucose levels are probably not rapidly reduced during activity due to the low hexokinase activity (Chapter III). A high concentration would then be required to establish a suitable concentration gradient between the plasma and intracellular compartments. Such a gradient permits the passage of glucose into the cell. Once glucose moves inside, the gradient prevents the leakage of glucose to the outside until it can be phosphorylated by hexokinase and acted upon by various other enzymes for storage as glycogen.

The liver glycogen level requires more time to return to resting levels after exercise than is required for repletion of muscle glycogen (Figures IV-3, IV-4), suggesting that the primary course of glycogen replenishment is first in muscle and then in the liver. this sequence is logical because muscle glycogen is utilized first during episodes of intense activity. The glycogen stored in the muscles is for use by muscles only,

whereas the liver acts as a storage site for glycogen to be shuttled to extrahepatic tissues at the onset of activity.

During intense activity the liver apparently releases free glucose into the bloodstream. However, the total amount of glycogen stored in muscles limits the amount of anaerobically produced work that occurs during intense activity. If an anaerobically dependent animal depletes part of its glycogen store through activity or fasting, it then reduces the amount of work that it can perform.

The same pattern of tissue glycogen utilization occurs during activity and recovery in fed and fasted Anolis (Figure IV-8). Fasted lizards, however, exhausted much faster than those that were fed. During activity, plasma glucose levels increase and then decline (Figure IV-5), a pattern during periods of glycogen depletion that produces a concentration gradient for glucose uptake by skeletal muscles. Muscle glycogen stores are not replaced more quickly in fasting versus nonfasting lizards (Figure IV-8). However the establishment of a plasma intracellular glucose gradient may allow for anaerobiosis to occur after a shorter recovery period (i.e., the free glucose entering the cell is used immediately for glycolysis).

Otner studies have shown that repetitive bouts of activity result in decreased times to exhaustion (Putnam 1979a, 1979b). Decreased glycogen stores are part of the reason for this reduction in performance. Lactate buildup would play a major role in performance reduction if lactate levels were still elevated. Anolis possesses the ability to quickly replace reduced muscle glycogen stores compared to mammals (Conlee et al. 1978). This ability may involve a mechanism that minimizes any potential reduction of activity resulting from fatigue or fasting. Muscle glycogen repletion appears to occur without the need for dietary carbohydrate intake.

A large part (approximately 60%) of an oral load of glucose is taken up preferentially by the liver in resting humans (Maehlum et al. 1977). Less than 15% ends up as muscle glycogen. However during post exercise recovery, a greater percentage of splanchnic glucose ends up as muscle glycogen. Muscle glycogen repletion has a higher priority over hepatic retention during the recovery period.

The picture is different during recovery in Anolis. Carbohydrate loading appears to increase glycogenesis. The ingestion of glucose after exercise causes an increase in hepatic glycogen. An oral glucose load has no initial effect on muscle glycogen. Skeletal muscle only shows an increase in storage after a second glucose feeding. This preferential overcompensation effect in liver tissue

suggests that muscle cells are storing glycogen at or near their maximum rate and further strengthens the conclusion that these animals rely heavily on stored glycogen in muscle for activity metabolism. Muscle tissue increases its storage of glycogen apparently only after saturating the hepatic glycogen storage rate. Whether this increase in storage is temporary is not known. If so, the only way the glycogen concentration can be reduced is through the catabolic activities of the muscle. Muscle cells do not possess glucose-6-phosphatase. Therefore, muscle glycogen cannot be utilized as a precursor to fat storage and can only be used by the muscle.

The storage of glycogen increases the time a lizard may be engaged in vigorous activity, but it will occur only if the lactate produced is not the maximum amount that can be tolerated. If not, the upper limit for lactate tolerance would prevent any increase in the time a lizard may engage in intense activity, regardless of the amount of glycogen stored.

The cost of using anaerobiosis coupled to gluconeogenesis is unfavorable. Consider that three high energy phosphate bonds are produced per glucose molecule utilized and derived from tissue glycogen. Six high energy phosphate bonds are used in the reconversion of glucose from pyruvate. Consequently, a net loss of three high energy

phosphate bonds results from coupling glycolysis with gluconeogensis. The loss in bond energy is equivalent to 21.9 kcal/mole of phosphate bond hydrolyzed.

The evolutionary benefits derived from an anaerobic system outweigh its costs. The metabolic machinery for aerobic metabolism is present although its performance is somewhat reduced (Chapter III). By utilizing glycolysis for the production of energy during activity Anolis is limited in the length of time it may engage in burst activity. Because of this limitation a large amount of stored energy is not required to engage in short term bouts of intense activity.

CHAPTER V
GLUCOSE TOLERANCE AND HORMONAL INFLUENCES IN GLUCOSE REGULATION

Introduction

The effects of insulin and glucagon have been examined in every order and suborder of reptiles except for rhyncocephalians (Penhos and Ramey 1973). The physiological effects are variable. Insulin is least effective in the lizards examined, and shows the most marked effect in alligators. Resting levels of plasma glucagon have not been determined in ectothermic vertebrates. However, injected glucagon raises the blood sugar level in all reptile species examined.

Glucose tolerance tests allow the effects of an added glucose load on body sugar levels to be evaluated. The usual procedure involves the intraperitoneal injection of a glucose solution and subsequent monitoring of blood glucose and/or tissue glycogen levels over time. Alligators show the greatest deviations from glucose homeostasis. This study is an attempt to characterize glucose homeostatis in green anoles and compare them to other species previously examined. Glucose tolerance, insulin and glucagon responses were used as criteria for this characterization.

Methods

Glucose Tolerance

Gastric glucose tolerance was examined during August. Large adult lizards (S-V length $\geq$ 600 mm), were purchased from an animal dealer. The lizards fasted for two days prior to testing (n = 70). Glucose was administered by gastric intubation at a concentration of 2 and 5 mg/g body weight. Plasma glucose and tissue glycogen were analyzed as described in Chapter II. Sugar concentrations were measured hourly for 10 hours, then 24, 48 and 72 hours after intubation. Plasma glucose is expressed as mg/dl $\pm$ 1 S.E.M. Skeletal muscle and liver glycogen are expressed as mg glycogen/g tissue $\pm$ 1 S.E.M.

Insulin

Mammalian insulin was obtained as a zinc salt from ICN Biomedical Inc. The insulin was injected intraperitoneally at concentrations of 1000 units/kg and 2000 units/kg. Animals were assayed for plasma glucose, muscle and liver glycogen at 10, 20, 30, 60, 90, 120, 300 minutes and 24 hours after the injection (n = 108). The results are expressed as previously described.

Glucagon

Mammalian glucagon was obtained from ICN Biomedical Inc. The hormone was injected intraperitoneally at concentrations of 10 ug/kg and 100 ug/kg (n = 108). Animals were assayed at the same intervals as in the insulin experiment. Results are expressed as previously described.

Results

Glucose Tolerance

Results are summarized in Figures V-1 and V-2. Plasma glucose levels peaked between 2 and 3 hours after ingestion (492 ± mg/dl). The plasma glucose levels were back to resting levels after 48 hours (135 ± 4.7 mg/dl). Muscle glycogen showed little effect of added glucose. Liver glycogen levels peaked between 10 and 24 hours (52.3 ± 3.33 mg/g).

Insulin

Insulin responses are summarized in Figures V-3 and V-4. In both cases there is a rapid onset of hypoglycemia. Plasma glucose levels dropped from 132 ± 2.1 mg/dl to 99 ± 1.4 mg/dl and 134 ± 1.7 mg/dl for the low and high insulin concentrations respectively. Within 60 minutes after the onset of hypoglycemia plasma glucose levels returned to normal values. At 90 minutes after administering insulin, the lizards showed a plasma

Figure V-1. Glucose tolerance for plasma glucose. Each symbol represents the mean of five animals.

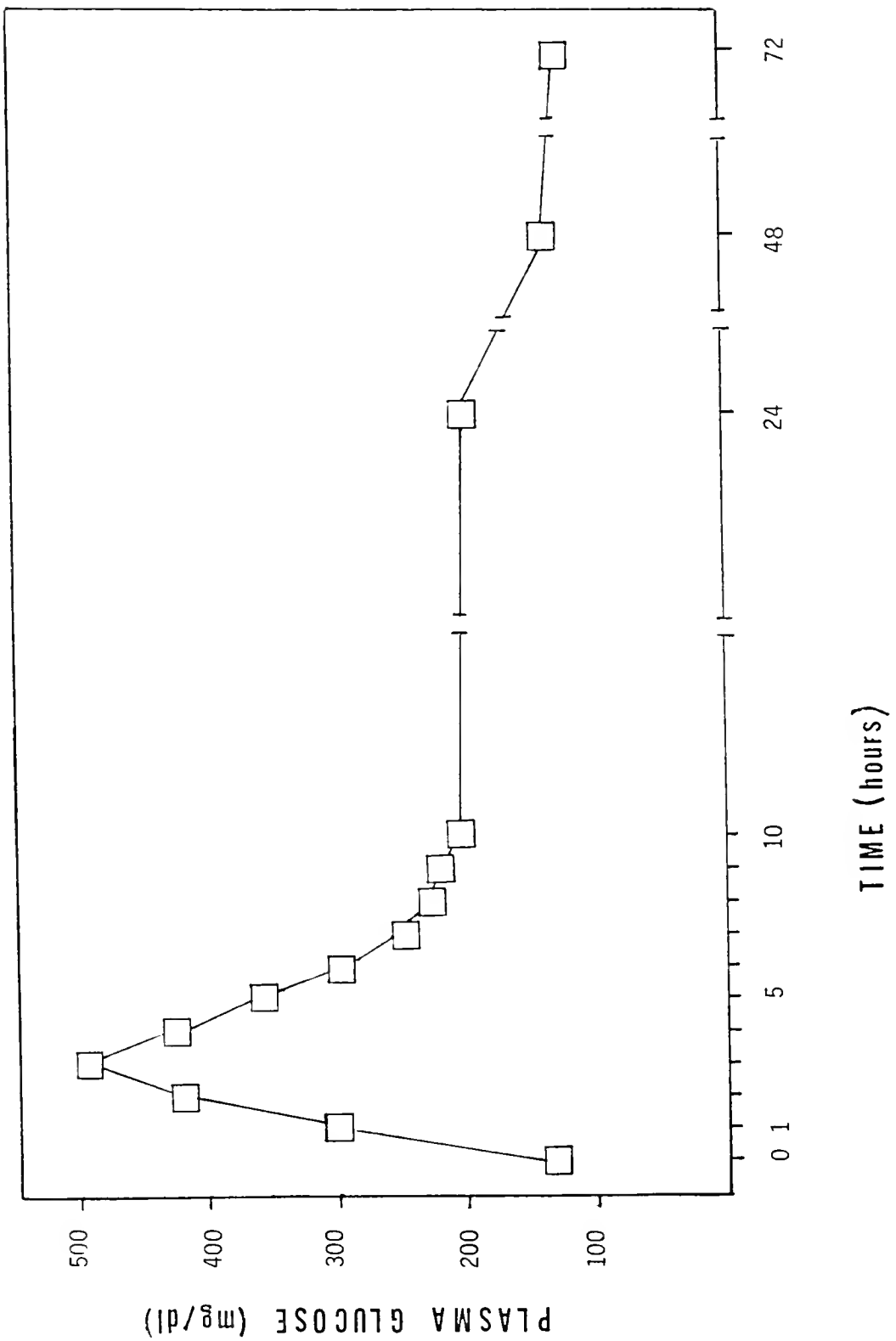

PLASMA GLUCOSE (mg/dl)
500
400
300
200
100
0 1
5
10
24
48
72
TIME (hours)

Figure V-2. Glucose tolerance for muscle and liver glycogen. Triangles = muscle glycogen. Circles = liver glycogen. Each symbol represents the mean of five animals.

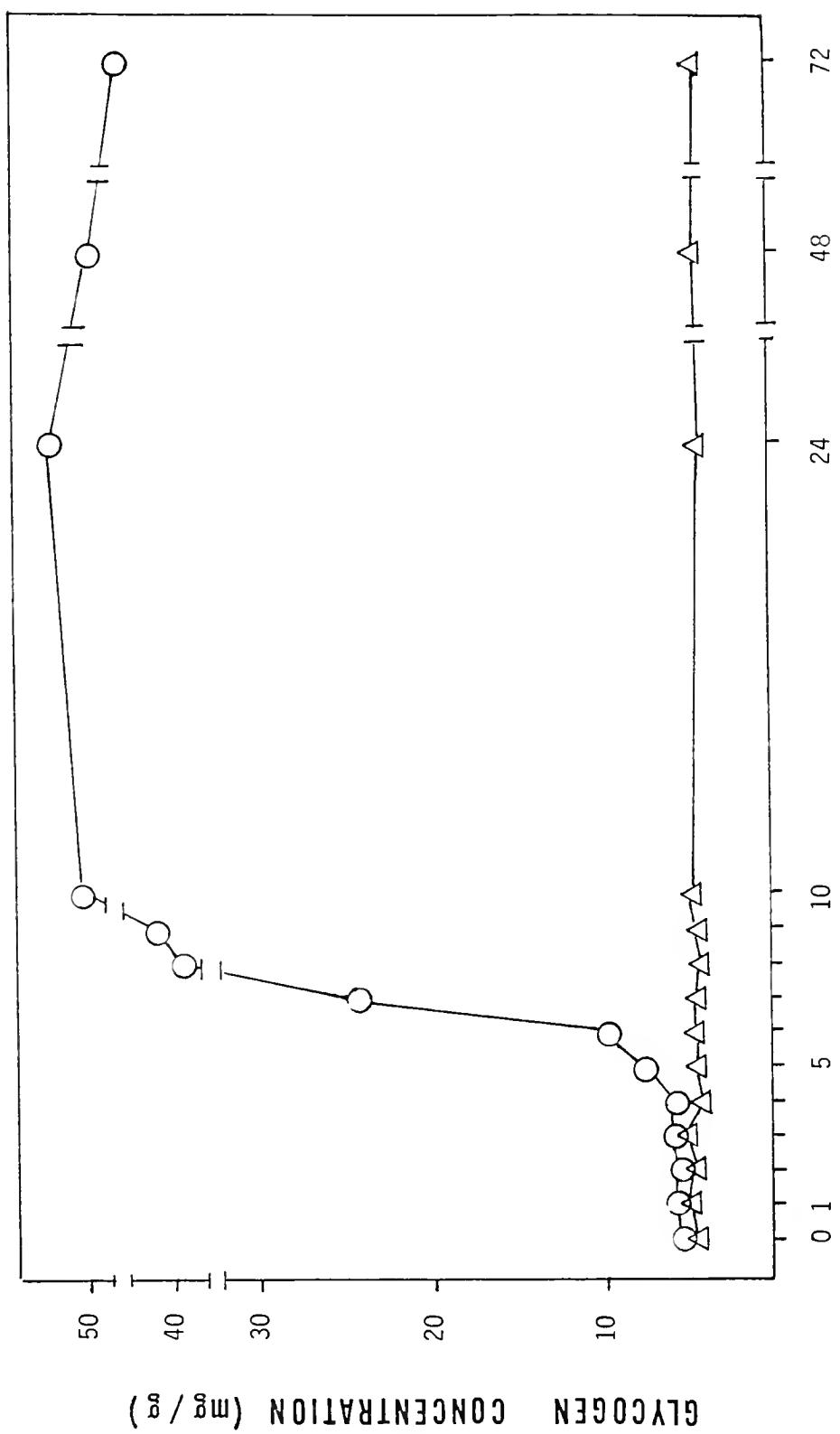

GLYCOGEN CONCENTRATION (mg/g)
50
40
30
20
10
0 1
5
10
24
48
72
TIME (hours)

Figure V-3. The effect of insulin on plasma glucose, muscle, and liver glycogen. Insulin concentration = 1000 units/kg. Squares = plasma glucose. Triangles = muscle glycogen. Circles = liver glycogen. Insulin was injected at time zero.

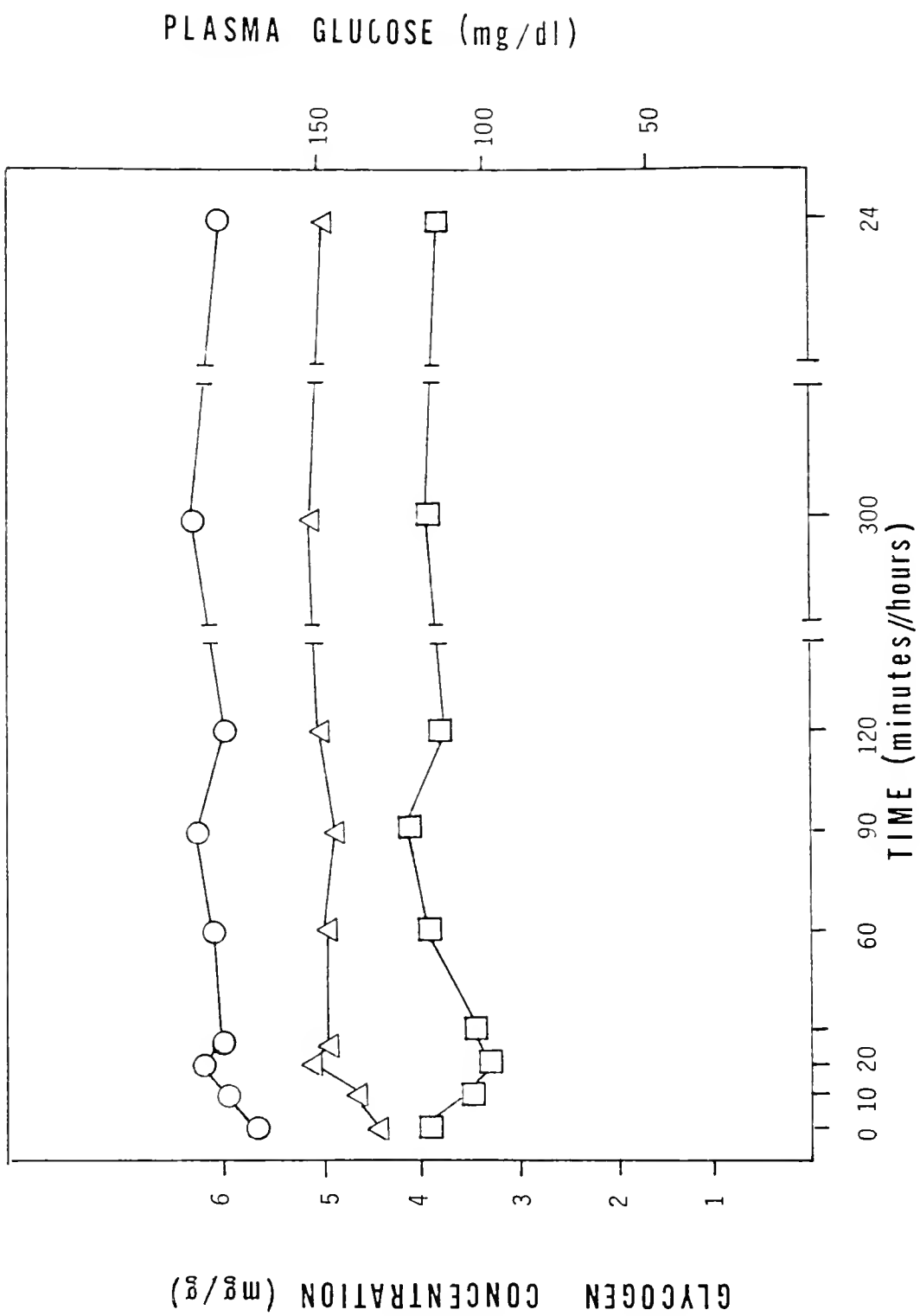
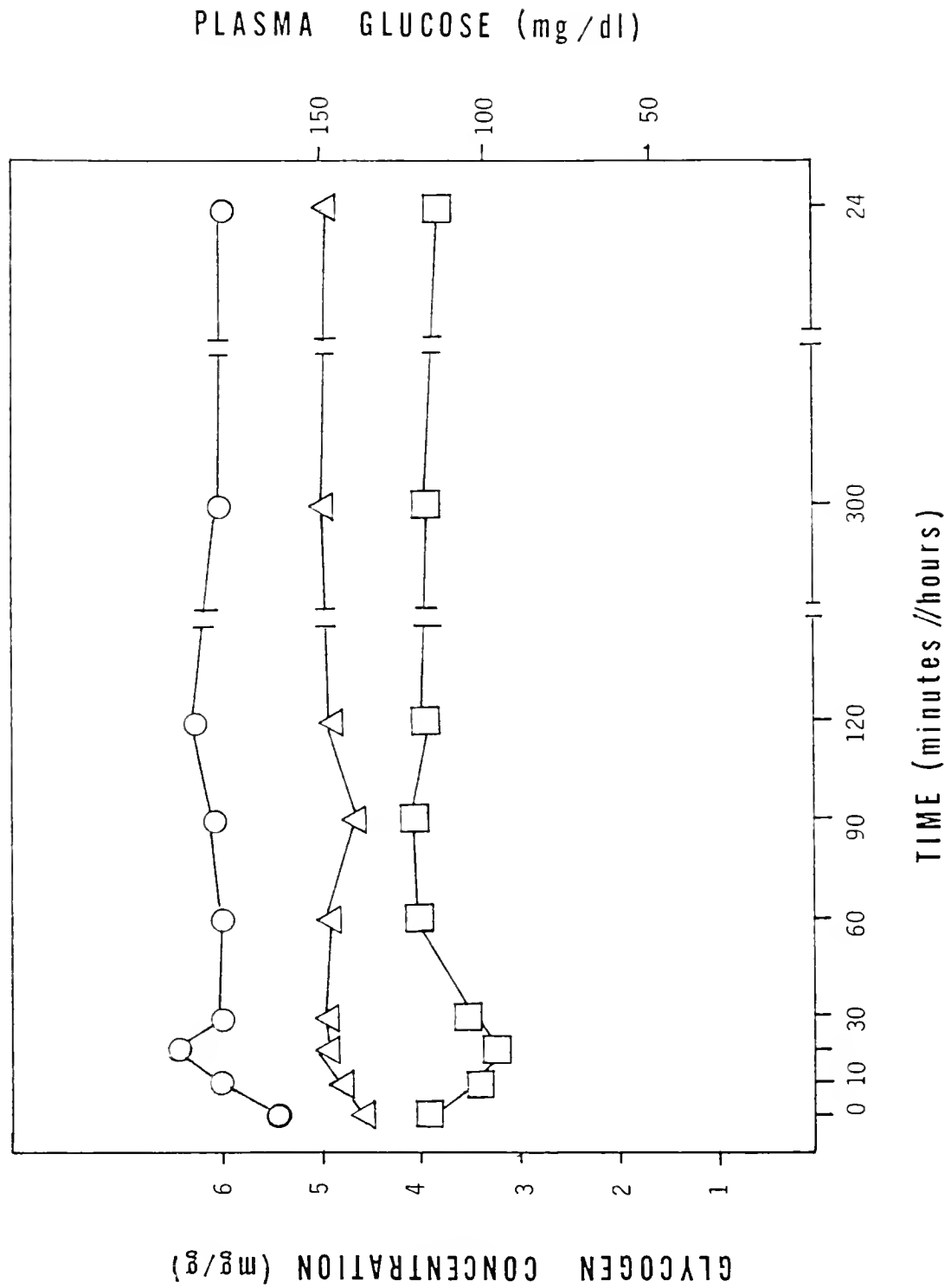

PLASMA GLUCOSE (mg/dl)
150
100
50
24
300
120
90
60
0 10 20
TIME (minutes//hours)
6
5
4
3
2
1
GLYCOGEN CONCENTRATION (mg/g)

Figure V-4. The effects of insulin on plasma glucose, muscle, and liver glycogen. Insulin = 2000 units/kg. Squares = plasma glucose. Triangles = muscle glycogen. Circles = liver glycogen. Insulin was injected at time zero.


PLASMA GLUCOSE (mg/dl)
150
100
50
GLYCOGEN CONCENTRATION (mg/g)
6
5
4
3
2
1
0 10 30 60 90 120 300 24
TIME (minutes //hours)

compensatory hyperglycemia which ended at 120 minutes. Muscle glycogen levels showed a rapid increase after the insulin injection. Within 10-20 minutes glycogen reached peak levels. Liver glycogen concentrations show the same trend, a rapid increase in stored glycogen followed by the maintenance of a relatively constant level. Both dosage groups produced the same results.

Glucagon

Glucagon responses are summarized in Figures V-5 and V-6. Animals injected with the higher concentration (100 ug/kg) showed the greatest effect. Plasma glucose concentration increased rapidly in both groups In the lower concentration group (10 ug/kg), this hyperglycemia continued for 6 hours. After 24 hours plasma glucose levels returned to pre-injection levels. In the 100 ug/kg group plasma glucose levels remained elevated for 48 hours. After 72 hours plasma glucose levels returned to normal. No effect of glucagon on muscle glycogen levels appeared in either group. In the liver stored glycogen levels decreased. In the 10 ug/kg group this reduction continued for 5 hours. Liver glycogen returned to resting levels after 24 hours (Figure V-5). In the higher concentration group, the reduction in liver glycogen continued for 24 hours. After 72 hours the glycogen levels returned to normal (Figure V-6).

Figure V-5. The effects of glucagon on plasma glucose, and muscle and liver glycogen. Glucagon concentration = 10 ug/kg. Squares = plasma glucose. Triangles = muscle glycogen. Circles = liver glycogen. Glucagon was injected at time zero.

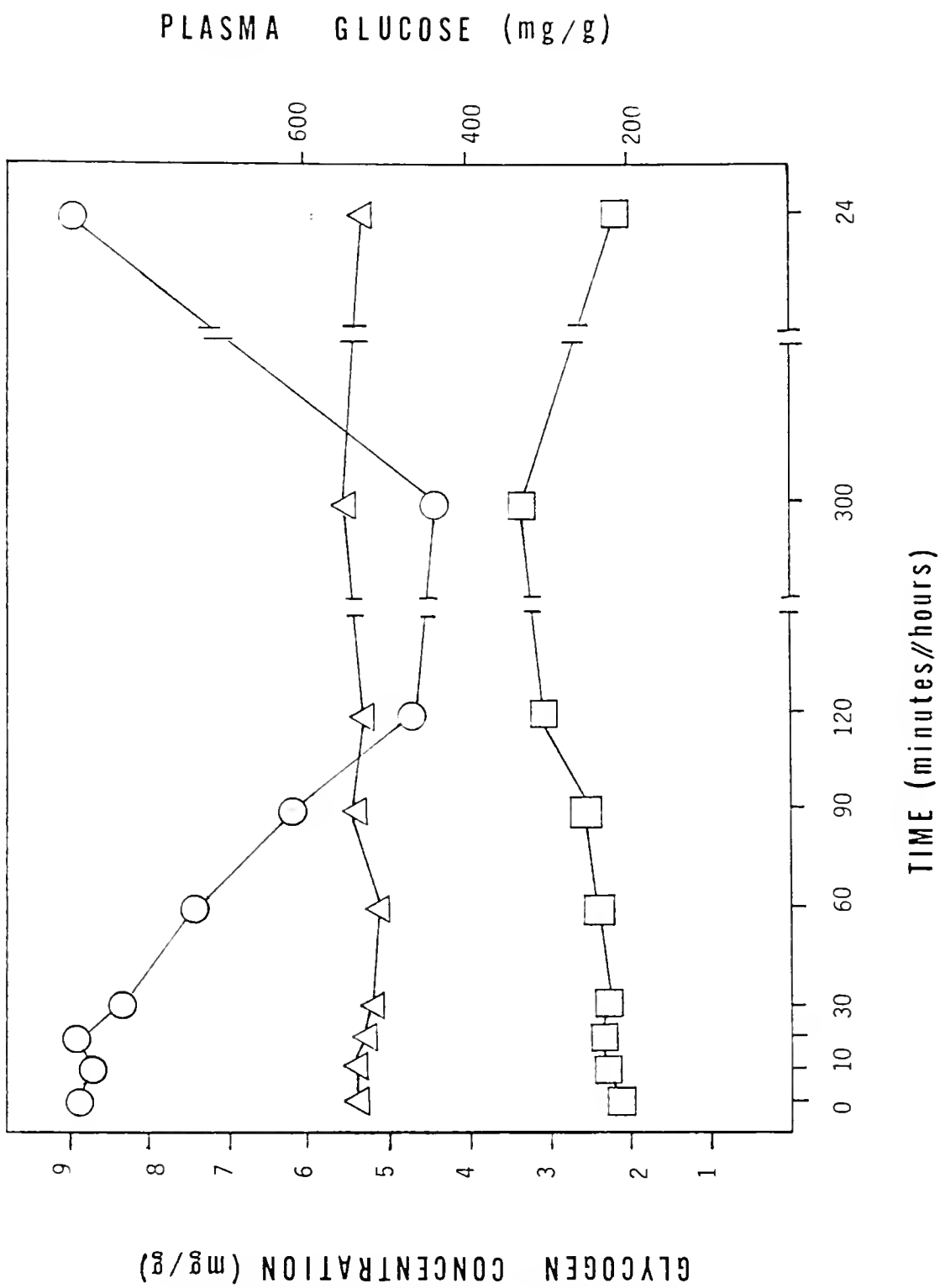
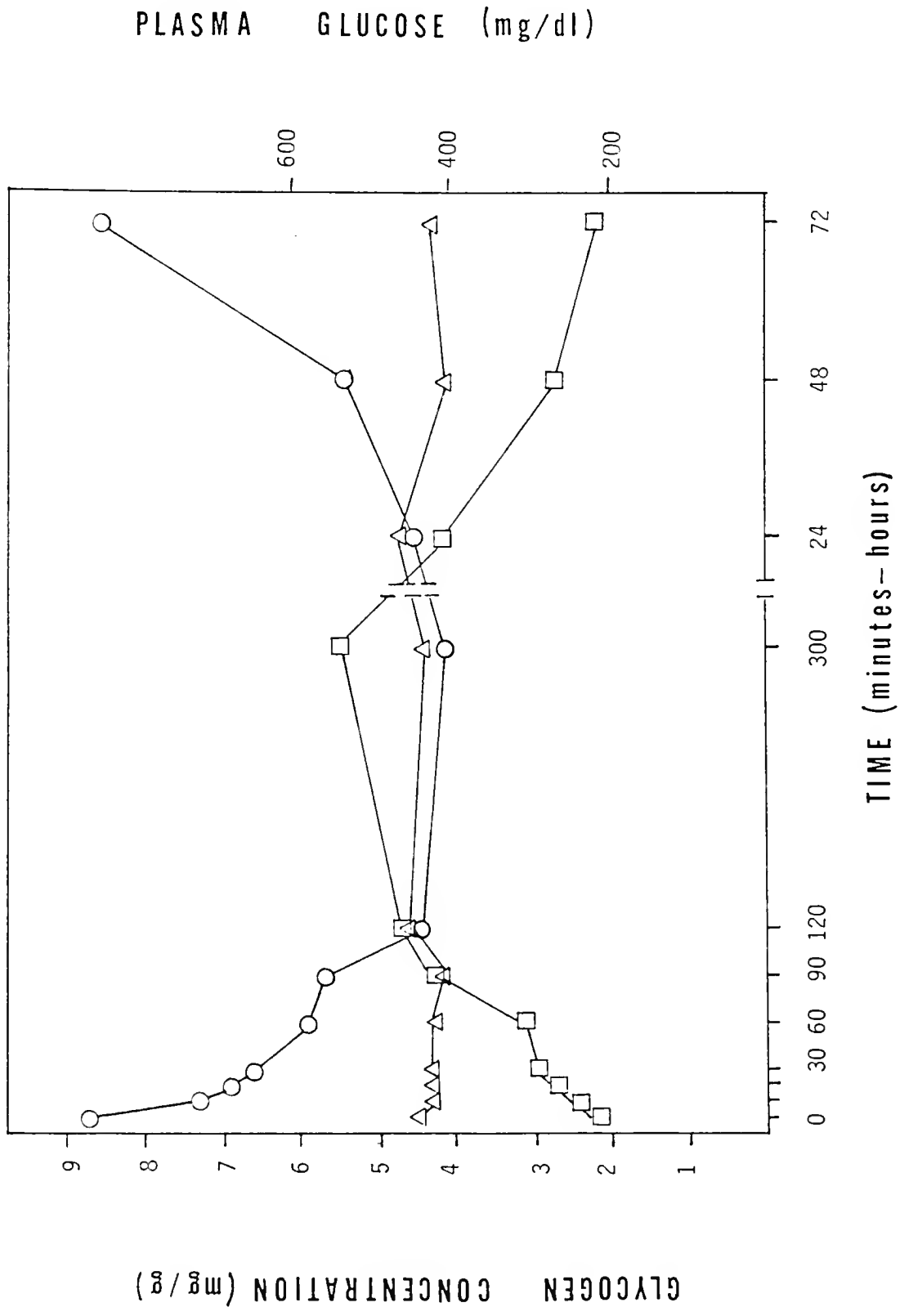

PLASMA GLUCOSE (mg/g)
600
400
200
24
300
120
90
60
30
10
0
TIME (minutes//hours)
GLYCOGEN CONCENTRATION (mg/g)
9
8
7
6
5
4
3
2
1

Figure V-6. The effects of glucagon on plasma glucose and muscle and liver glycogen. Glucagon concentration = 100 ug/kg. Squares = plasma glucose. Triangles = muscle glycogen. Circles = liver glycogen. Glucagon was injected at time zero.


PLASMA GLUCOSE (mg/dl)
600
400
200
TIME (minutes-hours)
0 30 60 90 120 300 24 48 72
GLYCOGEN CONCENTRATION (mg/g)
9 8 7 6 5 4 3 2 1

Discussion

Reptiles have a lower glucose turnover rate than do amphibians (Coulson and Hernandez 1953, 1964, Houssay and Penhos 1960, Penhos and Ramey 1973, Wright 1959, Miller and Wurster 1958) probably because amphibians are more sensitive to insulin (Penhos and Ramey 1973). Anoles appear to be similar to other reptiles. The relatively slow decline of plasma glucose after glucose loading may reflect a low hexokinase activity. However, other factors may be important including a low sensitivity to insulin.

Insulin does not appear to have a very significant effect in lowering plasma glucose. In fact, lizards as a group appear to be the least insulin-sensitive of all reptiles examined (Penhos and Ramey 1973). Anoles require a relatively high dosage of insulin to show the minimal signs of an insulin effect. This was also reported in other lizards (Miller and Wurster 1956). One interesting note is that although 1000 units/kg produced slight effects, 2000 units/kg caused typical insulin overdose symptoms, including trembling of the entire soma, muscle spasms and loss of muscle coordination.

Anoles have either an inherently low insulin production or they are not particularly sensitive to bovine insulin. The available literature has conflicting reports. Miller and Wurster (1958) reported that pancreatic beta cells are the site of insulin production in the lizard

Eumeces obsoletus. These animals like *Anolis*, were very glucagon sensitive. Pancreatectomized lizards become severely hypoglycemic, which suggests that lizards are very similar to birds in their dependence on glucagon for maintaining plasma glucose homeostasis.

Miller and Wurster (1958) reported beta cell destruction and a concomitant increase in blood glucose after alloxan treatment, suggesting that insulin production by these cells help to maintain blood glucose homeostasis. They reported earlier (1956) that glucose loading resulted in beta cell activation. However the hyperglycemia contined for at least 5 hours. They did not indicate how long it took for resting blood glucose levels to be achieved. Here it took 48 hours for resting blood glucose levels to be reached. If we assume the same or a similar situation metabolically, it is obvious that insulin sensitivity or production is low in lizards.

Consequently the combination of low hexokinase activity, low insulin production and low insulin sensitivity, a large glucose load cannot be efficiently handled. Confirmation must be made using radioimmunoassay.

Ozand and Narahara (1964) reported that insulin can increase the rate of glycolysis in the grass frog *Rana pipiens*, even in the absence of extracellular glucose. In addition to increasing cell membrane permeability, they suggested that insulin enhances the

activity of phosphofructokinase. If insulin can increase the rate of glycolysis, either directly or indirectly, then the low insulin production that occurs may be produced mainly for enhancement of the glycolytic pathway. I suggested in Chapter III that the regulation of high plasma glucose levels may be an adaptation allowing for the maintenance of a large blood-cell concentration gradient. In Anolis insulin may mainly increase glycolysis, but it will also aid the passage of glucose into the cells, regardless of how little insulin is produced.

Reptiles are very glucagon sensitive. After glucagon injections reptiles respond with hyperglycemia that may last from 1 to 10 days. Houssay and Penhos (1960) reported blood sugar levels increasing over 7 times resting levels after glucagon injections in a snake. A high sensitivity to glucagon may be an adaptation that allows lizards such as Anolis to maintain fairly high blood glucose levels. This view is supported by a histological examination of the lizard pancreas: lizards have a large proportion of alpna cells (Miller and Wurster 1956). Houssay and Penhos (1960) also reported that completely pancreatectomized lizards become severly hypoglycemic and hypothesized that after an insulin injection, pancreatectomized lizards should become hypoglycemic. Actually, such lizards showed an increase in blood glucose, which may

have been due to the use of an insulin preparation contaminated with glucagon. Nevertheless, glucagon has a much greater effect in lizards than does insulin.

The green anoles's reliance on burst anaerobic activity coupled with their inability to utilize glucose rapidly probably necessitate the need for glucagon sensitivity. If true, then lizards probably use glucagon as the primary means of plasma glucose homeostasis.

CHAPTER VI
SUMMARY AND CONCLUSIONS

Glucose regulation in Anolis is similar only in some respects to glucose regulation in mammals. Seasonal variation in liver glycogen and plasma glucose is greater in Anolis than in mammals. Much of the seasonal variation may be attributed to ultimately to hormones. Although plasma glucose shows a lot of variation, the levels maintained in Anolis are still higher than the levels maintained in mammals. The high plasma glucose levels are probably compensation for low hexokinase activity. Anolis probably cannot utilize glucose rapidly because of this low hexokinase activity. Maintaining high plasma glucose levels produce a large glucose concentration gradient between the outside and inside of the cells. Anolis apparently maintains this large gradient through a high glucagon sensitivity and a high glucagon production.

Enzyme activities reflect the use of anaerobiosis for energy production during activity. Anaerobiosis severely limits the duration of activity and is inefficient because of lactate production, but produces energy rapidly. Anolis most likely utilizes lactate by converting it to tissue glycogen. Because Anolis relys in many cases on burst activity, conversion of lactate to pyruvate for

aerobic catabolism would be inefficient as, aerobiosis occurs, but not at the level found in mammals. The lower level of aerobiosis is probably due to lower mitochondrial volume densities and membrane surface areas.

Carbohydrate loading after activity results in hepatic overcompensation. Only after supersaturating the hepatic system does muscle tissue start to store more glycogen than normal. Therefore muscle glycogen storage probably occurs at some preset rate very close to maximum. During the active times of the year, fasting is associated with a reduction in tissue glycogen. Glycogen is then more important as an energy source than fats. During the winter, when the animal may become torpid over parts of its range, fat reserves are more important than stored carbohydrates.

Glucose loading produces a long lasting hyperglycemia. This effect is most likely due to a combination of low insulin sensitivity, and/or production, and low hexokinase activity. Insulin only produces a response when administered in very high concentrations. The hypoglycemia produced is quickly compensated by a resulting hyperglycemia, probably as a result of glucagon secretion.

In other reptiles examined, the sensitivity to insulin is variable. Alligators are the most sensitive and possess low plasma glucose levels, while lizards are the least insulin sensitive and have high plasma glucose levels. In amphibians, anurans show insulin responses (i.e., long lasting hypoglycemia, Penhos and Ramey 1973). Anurans also have resting plasma glucose levels that are very low (ca. 25 mg/dl). Snakes are somewhat insulin sensitive and tend to have fairly low resting plasma glucose levels, 25 - 70 mg/dl (Penhos and Ramey 1973). It appears that insulin sensitivity is correlated with inherently low plasma glucose levels.

It seems certain that other terrestrial vertebrate ectotherms use variations on the themes presented here. Snakes possess low plasma glucose levels but tend to be less active than lizards. Anaerobic enzyme activities in snakes are similar to those of Anolis (Baldwin and Seymour 1977). Interesting enough, sluggish snakes with a high oxygen affinity appear better adapted for aerobic metabolism in muscle, while active snakes with lower oxygen affinity are better adapted for high rates of anaerobic energy production (Baldwin and Seymour 1977). Within a taxon, ectothermic vertebrates display variability in anaerobic and aerobic energy production.

The balance between aerobic and anaerobic metabolism needs to be examined further in other groups of ectotherms. The data presented here will hopefully provide the impetus for further research into the interrelationships involved in glucose regulation in lower vertebrates.

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APPENDIX
CALCULATIONS OF ENERGY EXPENDITURES FOR THE OXIDATION OF LACTATE AND GLUCONEOGENESIS

Gluconeogenesis

The following relationships are used to calculate the amount of phosphate bond energy and oxygen required to convert lactate to glucose. The costs of polymerizing glucose to glycogen are ignored. Three high energy phosphate bonds are required per lactate to make glucose-6-phosphate. The oxygen required for this process would be 10.6 ul per mole of lactate.

Oxidation

To completely oxidize one mole of lactate would require 3 moles of oxygen. This is equivalent to 67.2 ml per mmole of lactate.

Glycolysis

Two ATPs are synthesized per glucose molecule routed through glycolysis. Three ATPs are produced for each hexose derived from glycogen going through glycolysis. Therefore there are 1.5 moles of phosphate bonds produced per mole of lactate produced.

Aerobiosis

Produces a maximum number of 38 phosphate bonds per glucose cleaved from glycogen. This phosphate bond energy produced requires 6 oxygen molecules or 0.283 mmoles phosphate bond per ml oxygen used.

BIOGRAPHICAL SKETCH

Jerry McCoy was born on September 2, 1958, in Ocala, Florida. During his youth he became fascinated with animal life, especially reptiles. After graduating from Ocala Forest High School in 1976, he attended Central Florida Community College on a basketball scholarship. During the spring and summer of his freshman and sophomore years in college he worked at the Silver Springs Reptile Institute as a lecturer and venom lab technician. This experience reinforced his intrigue with herpetology. He received his B.S. in zoology from the University of Florida in 1981. Since then he has continued his studies on reptile physiology and ecology.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Harvey Lillywhite, Chairman
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. John Anderson
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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